SONOPANT DANDEKAR ARTS, V.S. APTE COMMERCE AND M.H. MEHTA SCIENCE COLLEGE, PALGHAR

Department of Biotechnology

PROJECT REPORT

TYBSC-BIOTECHNOLOGY

Academic Year 2022-2023

Prepared by

Department of Biotechnology

Sonopant Dandekar Arts, V.S. Apte Commerce and M.H. Mehta Science College, Palghar

INDEX

Sonopant Dandekar Shikshan Mandali's
SONOPANT DANDEKAR ARTS, V.S. APTE COMMERCE AND M.H. MEHTA SCIENCE COLLEGE, PALGHAR
Palghar, Dist – Palghar, Pin – 401 404, E – Mail: sdsmcollege@yahoo.com

Department of Biotechnology

NOTICE

Date: 23rd March 2023

Subject: Submission of Project Report

This is to inform you that all the **Third Year Biotechnology** students are required to submit the hard copy of your final project report by **06th April 2023**. All submissions should be made to the **Biotechnology Department** during office hours from **10:00 am to 02:00 pm**. Ensure your report is properly printed and bound.

Head of the
Biotechnology Department

(Dr. Kiran J.Save)
Principal

PRINCIPAL
Sonopant Dandekar Arts College,
V.S. Apte Commerce College &
M.H. Mehta Science College
PALGHAR (W.R.)
Dist. Palghar, Pin-401404

UNIVERSITY OF MUMBAI
No. UG/ 61 of 2018-19

CIRCULAR:-

Attention of the Principals of the affiliated Colleges and Directors of the recognized Institutions in Science & Technology Faculty is invited to this office Circular Nos. UG/126 of 2011, dated 13th June, 2011 relating to syllabus of the Bachelor of Science (B.Sc.) degree course.

They are hereby informed that the recommendations made by the I/c Dean, Faculty of Science & Technology at its meeting held on 8th June, 2018 have been accepted by the Academic Council at its meeting held on 14th June, 2018 **vide** item No. 4.38 and that in accordance therewith, the revised syllabus as per the (CBCS) for the T.Y.B.Sc. in Bio-Technology (Sem - V & VI), has been brought into force with effect from the academic year 2018-19, accordingly. (The same is available on the University's website www.mu.ac.in).

(Dr. Dinesh Kamble)
I/c REGISTRAR

MUMBAI – 400 032
6th ~~June~~ July, 2018

To

The Principals of the affiliated Colleges & Directors of the recognized Institutions in Science & Technology Faculty. (Circular No. UG/334 of 2017-18 dated 9th January, 2018.)

A.C./4.38/14/06/2018

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No. UG/ 61 -A of 2018 MUMBAI-400 032 6th ~~June~~ July, 2018

Copy forwarded with Compliments for information to:-

1) The I/c Dean, Faculty of Science & Technology,
2) The Director, Board of Examinations and Evaluation,
3) The Director, Board of Students Development,
4) The Co-Ordinator, University Computerization Centre,

(Dr. Dinesh Kamble)
I/c REGISTRAR

UNIVERSITY OF MUMBAI

Revised Syllabus for T.Y.B.Sc.
Programme- B.Sc.
Course- Biotechnology (USBT)
(Third Year – Sem. V & VI)

(Credit Based Semester and Grading System with effect from the academic year 2018-2019)

TYBSC Biotechnology Course Structure

Semester V

Course code USBT	Title	Theory /Practical	Marks	Credits	Nos of Lectures & Practical
501	Cell biology	Theory	100	2.5	60
502	Medical Microbiology & Instrumentation	Theory	100	2.5	60
503	Genomes and Molecular Biology	Theory	100	2.5	60
504	Marine Biotechnology	Theory	100	2.5	60
P501+502	Cell biology+ Medical Microbiology & Instrumentation	Practical	100	3.0	72
P503+504	Genomes and Molecular Biology+ Marine Biotechnology	Practical	100	3.0	72
Applied Component	Biosafety	Theory	100	2.0	48
	Biosafety	Practical	100	2.0	48
	TOTAL		**800**	**20**	**480**

Semester VI

Course code USBT	Title	Theory/ Practical	Marks	Credits	Nos of Lectures & Practical
601	Biochemistry	Theory	100	2.5	60
602	Industrial Microbiology	Theory	100	2.5	60
603	Pharmacology and Neurochemistry	Theory	100	2.5	60
604	Environmental Biotechnology	Theory	100	2.5	60
P 601-P 602	Biochemistry& Industrial Microbiology	Practical	100	3	72
P 603-P 604	Pharmacology - Neurochemistry and Environmental Biotechnology (50M)+ Project work (50M)	Practical	100	3	72
Applied component	Agribiotechnology	Theory	100	2.0	48
Applied component	Agribiotechnology	Practical	100	2.0	48
	TOTAL		**800**	20	**480**

Teaching pattern:

One (01) Credit would be of thirty- forty (30-40) learning hours; of this more than fifty percent of the time will be spent on class room instructions including practical as prescribed by the University. Rest of the time spent invested for assignments, projects, journal writing, case studies, library work, industrial visits, attending seminars / workshops, preparations for examinations etc. would be considered as notional hours. The present syllabus considers (60L as class room teaching and 15 lectures as Notional hours/ paper). Each lecture duration would be for 48 min

The names of the reference books provided in the syllabus are for guidance purpose only. Students and faculty are encouraged to explore additional reference books, online lectures, videos, science journals for latest/ additional information.

Examination pattern for:

Theory:

- The question paper for the Term End Exam would be of **100 marks** consisting of 5 Questions (20M each), of which one question would be common for all units in the syllabus.

- The question paper would be set for 150 marks including internal options.

- There shall be no internal exam for any paper.

Practical:

- Would be conducted over a period of 3 days; 50M each paper.

- Each student to perform 2 major and 2 minor practical for Sem V and 2 major and project presentation for Sem VI ,

- Viva would be conducted during the practical during Sem V; Sem VI would have ONLY project presentation

- Journals would be uniform throughout all the centres; matter would be communicated to all the centres by the syllabus committee.

- Distribution of marks for the experiments carried out during the examination:

  Sem V (50M/ paper): Major: 20M; Minor: 10M; Viva: 10M; Journal 10M.
  Sem VI (50M/paper): Major (x2): 40M; Journal: 10M; Project 50M

  The report could be around 25-30 pages with appropriate referencing and formatting.
  Marks distribution for the project would be as follows:
  25M documentation, 15M presentation, 10 M viva and interactions;

- Students would undertake a project for 1-2 months during the last semester for 50 M. The project **should** include **either** of the following:

  1. One/ more major instrumentation OR
  2. One / more major technique/s required in the field of interest OR
  3. Bioinformatics OR
  4. Biostatistics

Semester V

Course code USBT	Title	Unit	Topics	Credit	No of Lectures
501	Cell Biology	I: Cell cycle	Cell cycle Introduction: Prokaryotic and Eukaryotic- **3 Lectures;** The Early Embryonic Cell Cycle and the Role of MPF-**4 Lectures;** Yeasts and the Molecular Genetics of Cell-Cycle Control – **4 Lectures;** Apoptosis, Cell-Division Controls in Multicellular Animals- **4 Lectures**	2.5	15
		II: Cell Signalling	Cell signalling and signal transduction:Introduction General Principles of Cell Signaling - **3 Lectures;** Signaling via G-Protein-linked Cell-Surface Receptors - **3 Lectures;** Signaling via Enzyme-linked Cell-Surface Receptors - **3 Lectures;** Target-Cell Adaptation, The Logic of Intracellular - **3 Lectures;** Signaling: Lessons from Computer-based "Neural Networks"- **3 Lectures**		15
		III: Developmental Biology	Overview of how the modern era of developmental biology emerged through multidisciplinary approaches - **5 Lectures;** Stages of development- zygote, blastula, gastrula, neurula cell fate & commitment – potency- concept of embryonic stem cells, differential gene expression, terminal differentiation ,lineages of three germ layers, fate map - **6 Lectures;** Mechanisms of differentiation- cytoplasmic determinants, embryonic induction, concept of morphogen, mosaic and regulative development Pattern formation-- axis specification, positional identification (regional specification), Morphogenetic movements, Model organisms in Developmental biology - **4 Lectures**		15
		IV: Cancer Biology	Cancer: Introduction, Cancer as a Microevolutionary Process - **4 Lectures**; The Molecular Genetics of Cancer - **6 Lectures**; Cancer and Virus Cancer diagnosis and chemotherapy - **5 Lectures**		15
		Total			60

References:

1. Molecular Cell Biology. 7th Edition, (2012) Lodish H., Berk A, Kaiser C., K Reiger M., Bretscher A., Ploegh H., Angelika Amon A., Matthew P. Scott M.P., W.H. Freeman and Co., USA
2. Molecular Biology of the Cell, 5th Edition (2007) Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter. Garland Science, USA
3. Cell Biology, 6th edition, (2010) Gerald Karp. John Wiley & Sons., USA
4. The Cell: A Molecular Approach, 6th edition (2013), Geoffrey M. Cooper, Robert E. Hausman, Sinauer Associates, Inc. USA
5. Developmental Biology; Scott Gilbert; 9th Edition

Department of Biotechnology

Sr. No.	Roll No.	Name of the student	Title pf Project
1	95001	Pooja Yadav	Phytochemical Analysis of Methanolic ond Aqeous Extract of Swertiachirayita (Chirayita) and Tinosporacordifolia(Giloy)
2	95002	Tejas Kondhari	Study of In-Vitro Anti Inflammatory Activity of Some Local Herbs of Palghar District
3	95003	Khemchand Gaikwad	Screening of Bacterial Population from Farfantepenaeus Aztecus Species Collected From Vadrai Coast.
4	95004	Palak Bajpai	Isolation, Identification of Rhizobium From Root Nodules of Fenugreek Plants From Area Of Dahanu (Kosbad) And To Study Its Effect On Soil Fertility and Plant Growth.
5	95005	Prajyot More	Isolation of Lactobacillus From Different Curd Sample And To Check Its Probiotic Activity.
6	95006	Shushant Mali	Synthesis of Low Cost Bio-Adsorbents And Its Use In Industrial Waste Water Treatment
7	95007	Bhavesh Chothani	Analysis of Agricultural Soil Samples Taken From Palghar Vicinity
8	95008	Pooja Shirmade	To Study Phytochemical Analysis and Microbial Activity of Various Flowers of Tagetes, Chrysanthemum And Catharanthus Roseus on The Skin Flora.
9	95009	Riya Patil	Study of Phytochemical Analysis and Antimicrobial Activty of Aloe Vera And Formulation of Hydrogel
10	95010	Anushka Sankhe	In Vitro Anti-Microbial, Anti-Oxidant And Anti-Inflammetory Activities of Betal Leaf Extract.
11	95011	Shroti Topale	Comparative Nutritional Analysis of Different Milk Samples (Both Raw & Pasteurised Milk) By Qualitative And Quantitative Estimation.
12	95012	Nikita Rajapure	Development of Antimicrobial Fabrics Using Pigments Produced By Microbes Present In Marine Soil Found In Dahanu Cost.
13	95013	Shrishti Kushwaha	Isolation And Screening of Pigment Producing Bacteria From Dumping Yard of Boisar
14	95014	Sharukh Tadvi	Isolation of Dye Degrading Bacteria from Soil Samples Across Boisar Paint Industry Tal-Palghar Dist-Palghar, Maharshtra
15	95015	Amrita Yadav	Comparative Analysis of Phytochemicals And Antimicrobial Activity of Eucalyptus Leaves And Bark
16	95017	Helios Mascarnis	Study of Phytochemicals And Microbial Activityof Different Leaf Extracts From Murraya Koenigii (Curry Tree), Moringa Oleifera (Drumstick Tree) And Coriandrum Sativum (Coriander)

Sr. No.	Roll No.	Name of the student	Title pf Project
17	95018	Bushra Shaikh	Phytochemical And Antimicrobial Activity of (Neem) Azadirachta Indica.
18	95019	Dhanashree Satavi	A Comparative Study of Phytochemical Characteristics, Calcium And Protein Content of Borassus Flabellifer Fruit & Root.
19	95020	Ruchi Chaubey	Phytochemical Analysis of Beetroot Pigment for Formulation of Herbal Lipstick
20	95021	Manasi Raut	To Study the Phytochemicals Analysis and Microbial Activity of Phyllanthus Emblica and Vitis Vinifera
21	95022	Bhavesh Jain	Analysis of Fly Ash and Opuntia Ficus Indica as Efficient Textile Dye Adsorbent
22	95023	Aditi Shinde	Exploring The Potential of Banana Peel for Production of Ethanol.
23	95024	Vaishnavi Gupta	Comparative Analysis of Nutrient Composition of Milk From Different Breeds of Cows And Buffaloes
24	95025	Yash Tiwari	To Perform Phytochemical Analysis of Medicinal Plants (Tulsi & Neem) and Study Anti-Microbial Activity of Tulsi by Agar Cup Method.
25	95027	Mohammad Taukeer Kaliya	Comparative Analysis of Total Protein Content in Egg White Samples Collected from Different Locations.
26	95028	Sonali Dhangda	Comparative Study of Kiwi and Strawberry Fruit Samples from Palghar Local Market.
27	95029	Prathmesh Goli	Effect of Different Extraction Solvents on Antioxidant Activity, Phytochemicals and Protein Content of Marine Algae
28	95030	Yogesh Jha	Comparitive Analysis of Total Protein and Reducing Sugar Content in Processed and Freshly Squeezed Orange Juice
29	95031	Sanjana Singh	Formation Of Body Wash Using Antibacterial and Antioxidant Properties of Rose Petals Using Different Rose Varieties
30	95032	Swati Singh	Comparative Analysis of Total Protein and Reducing Sugar Content In Different Varieties of Grapes.
31	95033	Shimpi yadav	Phytochemical Analysis of Clitoria Ternatia Flower and Leaf

Teacher Signature

Head of the Department

Head of the
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Palghar (W) - 401 404

Sonopant Dandekar Shikshan Mandali's

SONOPANT DANDEKAR ARTS, V. S. APTE COMMERCE AND M. H. MEHTA SCIENCE COLLEGE

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Code.: (02525) 252163, Prin : 252317 • Resi.: 252316

website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com

(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 31 - 03 - 2023

CERTIFICATE

The work described in this project entitled "To Study the exploring Potential of Banana Peel for Production of ethanol."

"has been carried out independently by Aditi Rajesh Shinde Exam Seat no._____ with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
S. D. S. M. College,
Palghar (W) - 401 404

Signature of Principal

05/04/23

Signature of Examiner

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : _____

CERTIFICATE

The work described in this project entitled "COMPARATIVE ANALYSIS OF PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY OF EUCALYPTUS LEAVES AND BARK" has been carried out independently by MISS. AMRITA YADAV Exam Seat no. 4015413 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Signature of Principal

Head of the
Biotechnology Department
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S Patil 06/04/2023

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Ref. No. :

Date : 05/04/2023

CERTIFICATE

The work described in this project entitled "In Vitro Anti-Microbial, Anti-oxidant And Anti-inflammetory Activities of Betel leaf extract."has been carried out independently by Anushka Rajesh Sankhe Exam Seat no. 4015402 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

03/04/23

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
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05/04/2023

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Ref. No. :

Date : 05/04/2023

CERTIFICATE

The work described in this project entitled "ANALYSIS OF AGRICULTURAL SOIL SAMPLES TAKEN FROM PALGHAR VICINITY."

has been carried out independently by Bhavesh Ratilal Chothani Exam Seat no. 4015385 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

03/04/23

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
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(NAAC Reaccredated 'B' Grade)

Ref. No.:

Date : 31/03/23

CERTIFICATE

The work described in this project entitled "Analysis of Fly Ash and Opuntia Ficus Indica as Efficient textile dye Adsorbent"

"has been carried out independently by MR. BHAVESH DINESH JAIN Exam Seat no. with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

25/03/23
Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
S. D. S. M. College,
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Signature of Principal

6/4/23 6.4.23
Signature of Examiner

SONOPANT DANDEKAR ARTS, APTE COMMERCE, MEHTA SCIENCE COLLEGE
PALGHAR DIST. THANE

EXAMINER

Sonopant Dandekar Shikshan Mandali's

SONOPANT DANDEKAR ARTS, V. S. APTE COMMERCE AND M. H. MEHTA SCIENCE COLLEGE

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Ref. No. : Date : _____

CERTIFICATE

The work described in this project entitled "To Study phytochemical and Antimicobial Activity of Ethanolic Extract of Neem _____"has been carried out independently by Bushra Shaukat Shaikh Exam Seat no. 4015404 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
S. D. S. M. College,
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Signature of Principal

05/04/2023

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Ref. No. :

Date : _____

CERTIFICATE

The work described in this project entitled "A Comparative Study of Phytochemical Characteristics, Calcium & Protein Content of Borassus flabellifer fruit & Root. "has been carried out independently by Dhanashree Ganesh Salvi Exam Seat no. 4015403 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
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05/04/23

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website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com

(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : _____

CERTIFICATE

The work described in this project entitled "STUDY OF PHYTOCHEMICALS AND MICROBIAL ACTIVITY OF DIFFERENT LEAF EXTRACTS FROM MURRAYA KOENGII (CURRYTREE), MORINGA OLEIFERA (DRUMSTICK TREE) AND CORIANDRUM SATIVUM (CORIANDER)" has been carried out independently by HELIOS SANJAY MASCARNIS Exam Seat no. 4015397 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

29/03/2023.

Signature of Guide

Signature of H.O.D

Signature of Principal

Head of the
Biotechnology Department
S. D. S. M. College,
Palghar (W) - 401 404

DANDEKAR ARTS, APTE COMMERCE, MEHTA SCIENCE COLLEGE
PALGHAR
DIST. THANE

5/4/23.

Signature of Examiner

Sonopant Dandekar Shikshan Mandali's

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website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com

(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 31/03/23

CERTIFICATE

The work described in this project entitled "Screening of Bacterial Population from FARFANTEPNAEUS AZTECUS species collected from Vadrai coast."

"has been carried out independently by Mrs. KHEMCHAND BALU GAIKWAD Exam Seat no. 4015388 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

25/03/23

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
S. D. S. M. College.
Palghar (W) - 401 404

Signature of Principal

6.4.23

6/4/23.

Signature of Examiner

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 31-03-2023

CERTIFICATE

The work described in this project entitled "To study the phytochemical analysis and microbial activity of Phyllanthus emblica and vitis vinifera on the skin flora" has been carried out independently by Mansi Kalpak Raut Exam Seat no._____ with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

29/03/2023

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
S. D. S. M. College,
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Signature of Principal

05/04/2023

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 5/04/23

CERTIFICATE

The work described in this project entitled " Development of antimicrobial fabrics using pigment produced by microbes present in marine soil found in Dahanu Cost. "has been carried out independently by Nikita J. Rajpure. Exam Seat no. 95012 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
S. D. S. M. College,
Palghar (W) - 401 404.

Signature of Principal

05/04/2023

Signature of Examiner

SONOPANT DANDEKAR ARTS, APTE COMMERCE, MEHTA SCIENCE COLLEGE
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website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com
(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 31/03/2023

CERTIFICATE

The work described in this project entitled "ISOLATION, IDENTIFICATION OF RHIZOBIUM FROM ROOT NODULES OF FENUGREEK PLANTS FROM AREA OF DAHANU (KOSBAD) AND TO STUDY ITS EFFECT ON SOIL FERTILITY AND PLANT GROWTH" has been carried out independently by MISS. PALAK MAHESH BAJPAI Exam Seat no. 4015383 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

31/3/23.

Signature of Guide

Signature of H.O.D

Signature of Principal

Head of the
Biotechnology Department
S. D. S. M. College,
Palghar (W) - 401 404

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website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com

(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 31-03-2023

CERTIFICATE

The work described in this project entitled "To Study phytochemical analysis and microbial activity of various flowers of Tagetes, Chrysanthemum and Catharanthus roseus on the SKIN flora." has been carried out independently by Ms. Pooja Nagraj Shirmande Exam Seat no._____ with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

29/03/2023

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
S. D. S. M. College
Palghar (W) - 401 404

Signature of Principal

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 5/04/23

CERTIFICATE

The work described in this project entitled "Phytochemical analysis of Methanolic and aqueous extract of sweeta chirayita and Tinospora Cordifolia (Giloy)."has been carried out independently by Pooja devnarayan Yadav Exam Seat no. 401414 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
S. D. S. M. College,
Palghar (W) - 401 404

Signature of Principal

6.4.23 06/04/23

Signature of Examiner

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 31/03/2023

CERTIFICATE

The work described in this project entitled "ISOLATION OF LACTOBACILLUS FROM DIFFERENT CURD SAMPLE AND TO CHECK ITS PROBIOTIC ACTIVITY."

"has been carried out independently by MR. PRAJYOT RAJU MORE Exam Seat no. 4015398 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

31/03/2023

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Signature of Principal

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 5-04-2023

CERTIFICATE

The work described in this project entitled "EFFECT OF DIFFERENT EXTRACTION SOLVENTS ON ANTIOXIDANT ACTIVITY, PHYTOCHEMICALS AND PROTEIN CONTENT OF MARINE ALGAE"

"has been carried out independently by PRATHMESH PRADEEP GOLI Exam Seat no._____ with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 02/04/2023

CERTIFICATE

The work described in this project entitled "Study of Phytochemical Analysis And Antimicrobial Activity of Aloe Vera And Formulation of Hydrogel

"has been carried out independently by MS. RIYA · RAJENDRA · PATIL Exam Seat no. 4015399 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
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Palghar (W) - 401 404

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Signature of Examiner

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 5/04/23

CERTIFICATE

The work described in this project entitled "Phytochemical analysis of Beetroot Pigment for formulation of herbal lipstick"has been carried out independently by Ruchi Manibhushan Chaubey Exam Seat no._____ with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

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Biotechnology Department
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Palghar (W) - 401 404

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 5/4/23

CERTIFICATE

The work described in this project entitled "formation of body wash using Antibacterial & Antioxidant properties of Rose petals using Different Rose Varieties."

Sanjana. S. Singh "has been carried out independently by Sanjana. S. Singh Exam Seat no. 4015408 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
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05/04/2023

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Ref. No. :

Date : 05-04-23

CERTIFICATE

The work described in this project entitled "Isolation of Dye Degraeling Bacteria from Soil Sample Acros Boisar paint Industry

"has been carried out independently by Shahrukh · S. Tadvi Exam Seat no. 4015410 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

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S.S.Patil
05/04/2023

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Ref. No. :

Date : _____

CERTIFICATE

The work described in this project entitled "PHYTOCHEMICAL ANALYSIS OF CLITORIA TERNATIA FLOWER AND LEAF

"has been carried out independently by SHIMPI KAMLESH YADAV Exam Seat no. 4015415 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

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Palghar (W) - 401 404

Signature of Principal

06/04/23

Signature of Examiner

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 05/04/2023

CERTIFICATE

The work described in this project entitled "Isolation and Screening of pigment producing bacteria from Dumping yard of Boisar."has been carried out independently by Shrishti R. Kushwaha. Exam Seat no. 4015394 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 01/4/23

CERTIFICATE

The work described in this project entitled "COMPARATIVE NUTRITIONAL ANALYSIS OF DIFFERENT MILK SAMPLES (BOTH RAW & PASTEURISED MILK) BY QUALITATIVE & QUANTITATIVE ESTIMATION."has been carried out independently by SHRUTI · R · TOPALE Exam Seat no. 4015412 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Dilip P.
01/04/23
Signature of Guide

Dilip P.
Signature of H.O.D
Head of the
Biotechnology Department
S. D. S. M. College,
Palghar (W) - 401 404

Signature of Principal

S.P.Patil
05/04/2023
Signature of Examiner

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Ref. No. :

Date : 03/04/'23

CERTIFICATE

The work described in this project entitled "comparative Study of kiwi and Strawberry fruit Sample's from palghar local market

"has been carried out independently by sonali sitaram dhamgada Exam Seat no. 5015386 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

04/04/23

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
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Signature of Examiner

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 25-03-2023

CERTIFICATE

The work described in this project entitled " Synthesis of low Cost Bio-adsorbents and its use in industrial waste water treatment."

"has been carried out independently by Mr. Sushant Devendra Mali Exam Seat no. 4015396 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
S. D. S. M. College,
Palghar (W) - 401 404

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Signature of Examiner

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 5/04/23

CERTIFICATE

The work described in this project entitled "Comparative Analysis of total protein and reducing sugar content in different varieties of grapes."

Swati. R. Singh "has been carried out independently by Miss. Swati. Rajnish. Singh Exam Seat no. 4015409 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

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Head of the
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S. D. S. M. College,
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 05/04/2023

CERTIFICATE

The work described in this project entitled "[redacted] (Comprative Analysis of Total protein Content in Egg white Sample Collected from different locations)."

"has been carried out independently by M. TAUKEER . HUSENMIYA . KALIYA Exam Seat no. 4015392 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the Department
Biotechnology
S. N. S. M. College,
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 31/03/23

CERTIFICATE

The work described in this project entitled " Study of in-vitro anti-inflammatory activity of some local herbs of Palghar district"

"has been carried out independently by MR. TEJAS AVINASH KONDHARI Exam Seat no. 4015393 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

23/03/23

Signature of Guide

Dilpa. P

Signature of H.O.D

Signature of Principal

Head of the
Biotechnology Department
S. D. S. M. College,
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 03-04-2023

CERTIFICATE

The work described in this project entitled "Comparative analysis of Nutrient composition of milk from different breeds of cows and buffaloes"

has been carried out independently by Miss. Vaishnavi · P. Gupta Exam Seat no. 95024 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

03/04/2023
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 02/04/2023

CERTIFICATE

The work described in this project entitled "To perform phyto-chemical analysis of medicinal plants (Tulsi & Neem) and study Anti-microbial activity of Tulsi by Agar Cup method." has been carried out independently by MR. YASH. JYOTI. TIWARI. Exam Seat no. 4015411 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
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Signature of Principal

05/04/2023

Signature of Examiner

S. D. S. M. COLLEGE ★ PALGHAR ★ BIOTECH DEPT.

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : _____

CERTIFICATE

The work described in this project entitled "Comparative analysis of Total protein and Reducing sugar content in processed and freshly squeezed orange Juice." has been carried out independently by Yogesh mahesh maharaj Jha Exam Seat no. 95030 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Head of the
Biotechnology Department
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Signature of Principal

6.4.23 6/4/23

Signature of Examiner

SONOPANT DANDEKAR ARTS, APTE COMMERCE, MEHTA SCIENCE COLLEGE
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STUDY OF PHYTOCHEMICALS AND MICROBIAL ACTIVITYOF DIFFERENT LEAF EXTRACTS FROM *MURRAYA KOENIGII* (CURRY TREE), *MORINGA OLEIFERA* (DRUMSTICK TREE) AND *CORIANDRUM SATIVUM* (CORIANDER)

A Project Submitted To
The University Of Mumbai
Towards Partial Fulfilment Of The Degree Of
Bachelor Of Science In Biotechnology
Under The Guidance
Of
Mrs. Ishwari Mehta
(Assistant Professor Of Biotechnology Department)

Submitted By:
Helios Sanjay Mascarnis
Department Of Biotechnology
SDSM College, Palghar
T.Y.B.Sc Biotechnology (2022-23)

Sonopant Dandekar Shikshan Mandali's

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website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com

(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : _____

CERTIFICATE

The work described in this project entitled "STUDY OF PHYTOCHEMICALS AND MICROBIAL ACTIVITY OF DIFFERENT LEAF EXTRACTS FROM MURRAYA KOENGII (CURRYTREE), MORINGA OLEIFERA (DRUMSTICK TREE) AND CORIANDRUM SATIVUM (CORIANDER)" has been carried out independently by HELIOS SANJAY MASCARNIS Exam Seat no. 4015397 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

29/03/2023.

Signature of Guide

Signature of H.O.D

Signature of Principal

Head of the
Biotechnology Department
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PALGHAR
DIST. THANE

5/4/23.

Signature of Examiner

DECLARATION

I, **HELIOS SANJAY MASCARNIS** Student of T. Y. B.Sc. Biotechnology, hereby declare that the project entitled "Study of phytochemicals and microbial activity of different leaf extracts from *Murraya koenigii* (Curry tree), *Moringa oleifera* (Drumstick tree) *and Coriandrum sativum* (Coriander)." submitted by me for the academic year 2022-2023, is based on the actual work carried out by me under the guidance of **MRS. ISHWARI MEHTA**. I further state that this work is original and no part has been presented for any degree, diploma or similar title of any university.

ACKNOWLEDGEMENT

I am highly indebted and express my deepest sense of gratitude to my guide **Prof. Ishwari Mehta** and the Head of the department of Biotechnology, **Dr. Shilpa Gharat** for their valuable guidance and suggestions throughout the research work and preparation of this project.

They have been very helpful by giving constructive encouragement in spite of their busy schedules. Due to their guidance and constant interest, the project could be completed well in time. I would also like to acknowledge the constructive and valuable help of various people during the process of completion of the present research work.

I would like to expresses gratitude to the Principal and management of Sonopant Dandekar Arts, V.S.Apte commerce and M.H. Mehta science college, Palghar for permitting to conduct the project work in the institute.

I would also like to thank other professors, **Prof. Shailaja Palan, Prof. Runali Raut, Prof. Apurva Save, Prof. Sajari Raut, Prof. Riddhi Bisht, Prof. Niyati Patil, Prof. Lisa Sam, Prof. Hardik Churi.** Lab assistant **Jidhnaya Patil** and lab attendant **Nitin Jadhav, Nitesh Pagi, Bhavesh Bhoir and Bhupendra Raut and Suvidha Jadhav** for their immense guidance and concern through out my project work and for helping wherever required.

Finally, I wish to extend a warm thanks to everybody involved directly and indirectly with my work.

Contents

Abstract

Phytochemicals are chemicals synthesised by plants and are magical to say the least. They have uses ranging from creation of pesticides as well as traditional medicine. They can be extracted by various chromatographic techniques and put into use. In the current skincare market, products that have natural composition are gaining relevance solely due to ayurveda and almost zero side effects they result in. In order to test the microbial property of various leaf extracts conferred by the underlying phytochemicals, leaves from *Murraya koenigii* (Curry tree), *Moringa oleifera* (Drumstick tree) *and Coriandrum sativum* (Coriander) were dealt with in this study. Three solvents, namely ethanol, chloroform and water were used to make solvent extracts. The microbial activity of these solvent extracts was tested on an isolated skin microbial colony by the use of agar cup diffusion method. A control plate containing the three solvents and Gentamicin was incubated alongside the test plates. The test plates were later compared with the control plate and their zone of inhibition was measured and noted. All chloroform leaf extracts and ethanol extracts of drumstick leaves showed zone of inhibition. Thin layer chromatography was performed and the retention factor was noted. In fresh extracts, coriander leaves scored the highest (0.431) and in dry extracts curry leaves scored the highest retention factor value (0.282). Phytochemical assay of the leaf extracts were later done. Alkaloids were the most prominent phytochemicals of the bunch.

Key words: Phytochemicals, Leaves, Solvents, Zone of inhibition, Agar cup diffusion method, Phytochemical assay

Introduction

Plants have been admired, cultivated and used for centuries for their microbial activity on skin pathogens. This activity is a result of various phytochemicals present in them. To name a few, tannins, flavonoids, phytosterols are some of these phytochemicals. Phytochemicals generally originated from the plant source are nothing but the bioactive compounds also known as secondary metabolites. There are two types of metabolites produced in plants viz. Primary metabolites and Secondary metabolites. Primary metabolites are important for the plants regular metabolism such as growth and development. Secondary metabolites produced by plants may have little need for them. These are synthesized in almost all parts of the plant like bark, leaves, stem, root, flower, fruits, seeds, etc. During past several years, phytochemicals have been used worldwide as the traditional herbal medicine. Because of this pharmaceutical industries as well as researchers put a greater emphasis on the phytochemical studies. Also these phytochemicals present in the different plant parts are used up by the local people for healing of certain disorders. [Ugochukwu et al. (2013)]

CURRY LEAVES

Curry leaves biological name is *Murraya koenigii*, which is also known as "Kari patta" or "Meethineem" in the local dialect. Curry Leaves belongs to Family Rutaceae (that mainly have approximately 150 genera and 1500 species [Kedarnath et al. (2013)]. *Murraya koenigii* is found to be native mainly to India and Sri Lanka. Additionally, it can be found in some other South Asian countries as well. It is a deciduous, small tree or a shrub which is aromatic in nature and grows up to a height of about 6-9m and up to an altitude of 1500m. It is an important ingredient in Indian food owing to its fragrance and aroma. This plant is known to be the richest source of carbazole alkaloids. [Nandita Kamat et al. (2015)]. Curry leaves are therapeutic agents and effective in inhibiting Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Klebsiella pneumonia, Salmonella typhi, and Bacillus subtilis which are comparable to commercial antibiotics chloramphenicol, streptomycin, and gentamicin. Antioxidant protein from curry leaves showed antibacterial activity against pathogenic bacteria. [B. Mylarappa et al. (2010]

DRUMSTICK

According to [Dr.Vanisha S. Nambiar (2023)]. The Moringa or Drumstick tree (aka) Munaga, Muruggai, Muranka) is perennial, erect, slender, medium-sized with many arching branches. It has drumstick-like fruits, small white flowers and small and tear- drop shaped round leaves, which are cooked and eaten as vegetable. There are a number of preparations made from Moringa trees and exported from India, namely fresh drumstick fruit, Drumstick powder, Moringa oil, Moringa seed, Moringa leaf powder, Moringa leaf, Moringa pickle, Moringa tea powder, Moringa fruit powder, Moringa seed kernel, Moringa cake powder and Moringa root and many more. In Southern parts of India, Moringa leaves are used to prepare a pulse preparation called sambar. Drumstick is also preserved and exported worldwide. Tender drumstick leaves, finely chopped, make an excellent garnish for any vegetable dishes, dals, sambars, salads, etc. Drumstick leaves are also rich sources of flavonols such as kaempferol and 3'-OMe quercetin. A flavone, acacetin and a glycoflavone 4-OMe Vitexin was also identified. The phenolic acids identified included melilotic acid, p-coumaric acid, and vanillic acid [Nambiar et al. (2005)]. Quercetin also shows antiviral activity.

CORIANDER

Coriandrum sativum belongs to the family Umbelliferae, is a vertical annual herb with a definite taproot, having diverging shoots ranging from 20 cm to 70 cm in height. Leaves of *Coriandrum sativum* are green or dark green, lanceolate, having plain surfaces mutually and are flexible in form and lobed. Crop of *Coriandrum sativum* reaches its blossom stage within 45 to 60 days after disseminating and ripens within 65 to 120 days, all depends on diversity that what was the condition of cropping. [Saeed, (2007)].

Coriandrum sativum is extremely believed medicinal ayurvedic tree generally recognized as Dhanya. Vital oil, fatty acids, flavonoids and sterols have been cultivated from diverse portions of C*oriandrum sativum*. Different parts of this plant such as leaves, seeds, flower and fruit, have different activities such as antifungal activity, antioxidant, anti-diabetic, anti-helmintic, anti-mutagenic soporific-hypnotic, diuretic, anticonvulsant, lowering cholesterol, anti-feeding, anticancer, anxiolytic, hepatoprotective, anti-ulcer, anti-protozoal, defensive role counter to poisonousness of lead, detoxification of heavy metal and post-coital [Zare-Shehneh (2014)]

Rationale

In ancient times, skin injuries and infections were combated with pure plant extracts. These were generally rubbed upon or held against the affected area for application. Cut to the present times, there has been a surge in skin products composing of plant extracts. Undeniably and scientifically backed, certain plant leaf phytochemicals have been proved to exert either inhibitory or exhibitory activity. We need to study this inhibitory activity of plant extracts for its use in the sole destruction of pathogenic bacteria in-case of a wound formation. Similarly studying the exhibitory activity can help us estimate better formulate certain media for the isolation and study of such organisms. These efficiencies need to be compared with established standards. Such experimental results can be used by the general population to end their over-reliance on chemical based skin products by choosing plant based skin products as well as by skin product manufacturers to modify the composition of their products in order to combat a pathogen that's quite resistant to the current formulation.

Review of Literature

There's a huge cultural shift towards natural products packed with antimicrobial properties and the world is shifting away from chemically constituted counterparts. Phytochemicals are the main active components that show antimicrobial activity. Phytochemicals are present in leaves and some of them are more economical to grow than to synthesize chemicals from scratch. After realizing the unfathomable strength of such phytochemicals, various researchers began to study various phytochemical sources such as leaves of different plants and assayed the phytochemicals present in them by usually employing solvent extraction processes.

The study of phytochemicals present in leaves were done by several authors:

- Piyush Kashyap et al. Recent Advances in Drumstick (Moringaoleifera) Leaves Bioactive Compounds: Composition, Health Benefits, Bioaccessibility, and Dietary Applications, *Antioxidants* 2022

  This review emphasizes on the chemical background of phytochemicals and other bioactive compounds of Drumstick tree (Moringaolifera) and ties them up with health benefits like antimicrobial activity, antioxidant activity, anti-cancerous activity, anti inflamatory activity and much more. The author provided tables showing the extact processes to procure the bioactive compounds responsible for them. The subject of bioaccessibility and bioavalibility is broached too. Overall a great amalgamation of all research associated with bioactive compounds present in the drumstick tree.

- Dr.Rupali Rajvanshi et al. Phytochemical Analysis of Curry Leaves; 367; Volume 8 Issue 9, September 2019

  This analysis was carried out to detect the Phytochemical content in curry leaves . They used solvent extraction processes to extract the phytochemicals from curry leaves. The solvents used were ethanol, methanol and water. The extracts were tested for the presence of Alkaloids, Flavonoids, Glycosides, Steriods, Cardiac glycosides, Saponins, Phenols, Tannins, Terpeniod, Quinone, Amino Acids and Proteins. They even graded the amount of phytochemicals present in the sample into three levels namely: Present, Moderately present and Appreciable amount. Overall a good research covering a broad amount of phytochemicals.

- Shreyasi Mallik et al. Phytochemicals of Coriander, Cumin, Fenugreek, Fennel and Black Cumin: A Preliminary Study; Volume 45

  This analysis was carried out to detect the Phytochemical content in Coriander, Cumin, Fenugreek, Fennel and Black Cumin. They used solvent extraction processes to extract the phytochemicals. The solvents used chiefly was ethanol. The extracts were tested for the presence of Alkaloids, Flavonoids, Steriods, Saponins, Tannins, Coumarin and Anthrocyanin. They graded the amount of phytochemicals present in the sample into four levels namely: Highly present, Moderately present, Present and Not present. Overall a good research covering a broad amount of samples.

Aim and Objectives

Aim: To study the microbial activity and phytochemicals of various plant leaf extracts by agar cup method, thin layer chromatography and various phytochemical tests.

Objectives:

1) To isolate and study colony characteristics of skin microflora

2) To check the microbial activity of leaf extracts on the isolated skin microflora

3) To carry out analysis of phytochemicals by chemical assay.

4) To separate phytochemicals by thin layer chromatography.

5) To calculate the retention factor of leaf extracts using thin layer chromatography.

Materials and Methodology

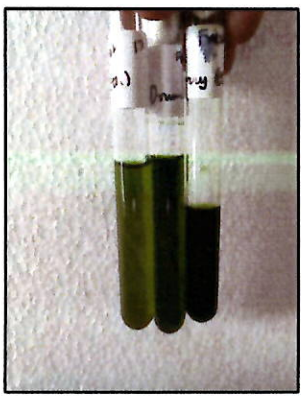
For extract preparation:

Type	Particulars	Quantity
Sample	Coriander leaves Curry Leaves Drumstick Leaves	10 gm 10 gm 10 gm
Glassware	Clean and dry test tubes Clean and dry centrifuge tubes Clean and dry pipettes (1ml) Pasteur Pipettes	9 units 9 units 6 units 6 units
Chemicals	Chloroform Absolute Ethanol	15 ml 15 ml
Miscellaneous	Distilled water Mortar and pestle Rotary Shaker Microwave oven Centrifuge at 3000 rpm for 15 minutes	15 ml 1 unit 1 unit 1 unit 1 unit

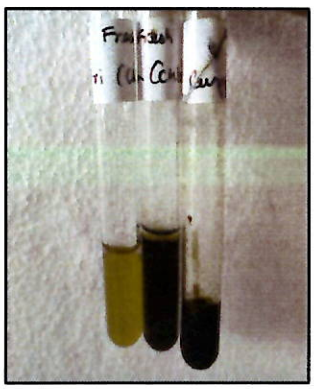
Method: For fresh leaf extract:

1 gram of leaf sample was weighed and later ground using mortar and pestle. The ground content was added to test tubes. 5ml of ethanol, chloroform and water was added in separate test tubes all containing the ground content. This procedure was repeated for each type of leaf. These tubes were then subjected to a rotary shaker overnight. The liquid phase of these tubes were later transferred to centrifuge tubes which were later centrifuged at 3000 rpm for 15 minutes. A Pasteur pipette was used to transfer the plant extract (supernatant) into labelled test tubes.

Ethanol extracts



Chloroform extracts



Water extracts

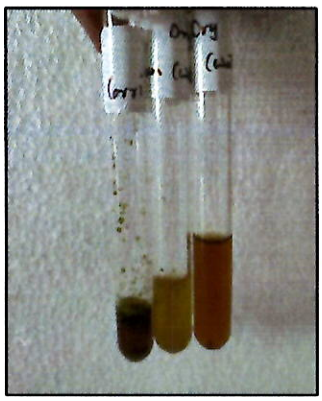
For dry leaf extract:

The leaf sample was microwaved at ~100^{0}C for 2 minutes (with regular scraping and turning) The dried content was hand-crushed and 1 gram of this dried content was weighed and added to test tubes. 5ml of ethanol, chloroform and water was added in separate test tubes all containing the dried content. This procedure was repeated for each type of leaf. These tubes were then subjected to a rotary shaker overnight. The liquid phase of these tubes were later transferred to centrifuge tubes which were later centrifuged at 3000 rpm for 15 minutes. A Pasteur pipette was used to transfer the plant extract (supernatant) into labelled test tubes.

Ethanol extracts

Chloroform extracts

Water extracts



For Culture inoculation and isolation:

Type	Particulars	Quantity
Media	Sterile nutrient agar plate	1 unit
Miscellaneous	Nichrome loop Sterile saline Incubator at 37^{0}C for 24 hours	1 unit 10 ml 1 unit

Media used: Sterile Nutrient agar
Composition: In gram/L
Beef extract: 3.0g
Peptone: 5.0g
Agar: 20.0g
Distilled water: 1000ml
Final pH: 7.0 – 7.2

Method:

Test culture from skin was collected using a nichrome loop. The loop culture was inoculated in 10ml sterile saline. The culture was later streaked by using quadrant method on a sterile nutrient agar plate. The nutrient agar plate was then incubated at 37°C for 24 hours.

For Gram staining & Microscopic Visualization:

Type	Particulars	Quantity
Glassware	Clean, dry and grease-free glass slide Clean and dry droppers	1 unit
Reagents	Gram's crystal violet Gram's iodine Safranin 95% Alcohol	~2 ml ~2 ml ~2 ml ~5 ml
Miscellaneous	Distilled water Compound microscope (100x) Cedar Wood oil	25 ml 1 unit 1 drop

Method:

Make a smear on a clean and dry glass slide with the microbial suspension. Heat fix the smear and air dry it. Add 1-2 drops of crystal violet dye on the smear and let the slide rest for 1 minute. Wash the slide with a gentle indirect stream of water for 2 seconds. Add mordant on the smear (Gram's iodine) and wait for 1 minute. Repeat the gentle water wash and decolorize the slide by using a few drops of alcohol. Add some safranin to the smear and wait for 1 minute and repeat the gentle water wash step. Let the slide air dry or tilt it on a coarse filter paper. Add a drop of cedar wood oil on the smear and view under the 100x (oil immersion) lens of a compound microscope.

For Agar cup method:

Type	Particulars	Quantity
Test Culture	Saline containing isolated colony	1 unit
Media	Sterile nutrient agar plate	4 units
Glassware	Beaker Sterile pipettes (1 ml) Petri dish	1 unit 9 units 1 unit
Antibiotic	Gentamicin	1 ml
Chemicals	Absolute Ethanol Alcohol Chloroform	20 ml 20 ml 1 ml
Miscellaneous	Distilled water Sterile cork borer Sterile cotton swab Refrigerator at 4^0C Incubator at 37^0C for 24 hours	1 ml 1 unit 3 units 1 unit 1 unit

Method:

For loading plant extracts:

Pour about 20 ml of molten nutrient agar in each sterile petri plate and allow them to solidify. Using a sterile cotton swab the test culture was swabbed on the surface of the media. A sterile cork borer should be used to make wells in the agar plates. The prepared plant extracts were loaded into these wells. The plates were placed in an incubator at 37^0C for 24 hours. The zone of inhibition was recorded.

For loading controls:

Pour about 20 ml of molten nutrient agar in a sterile petri plate and allow it to solidify. Using a sterile cotton swab the test culture was swabbed on the surface of the media. A sterile cork borer should be used to make four wells in the agar plates. Absolute ethanol, Gentamicin, sterile distilled water and chloroform were loaded into these four wells. The plates were placed in an incubator at 37^0C for 24 hours. The zone of inhibition was recorded.

For Testing Phytochemicals:

Type	Particulars	Quantity
Glassware	Clean and dry test tubes	16 units
Chemicals	Dragendorff's reagent Concentrated H_2SO_4 10% ferric chloride solution	~1 ml ~1 ml ~1 ml
Miscellaneous	Droppers	3 units

Method:

For detection of Alkaloids:
Dragendorff's test: Add few drops of plant extract and add 1 to 2 drops of Dragendorff's reagent into a test tube. A reddish brown precipitate indicates presence of alkaloids.

For detection of Flavonoids:
Concentrated H_2SO_4 Test: Add few drops of plant extract and add 3 to 4 drops of concentrated H_2SO_4 into a test tube. Production of orange colour indicates presence of flavonoids.

For detection of Tannins:
Tannins test: Add few drops of plant extract and add 3 drops of 10% ferric chloride solution into a test tube. Blue-green colouration indicates the presence of tannins.

For detection of Phytosterols:
Salkowski's test: Add few drops of plant extract and add few drops of concentrated H_2SO_4 in a test tube. Red coloured layer formation indicates the presence of phytosterols.

For Thin Layer Chromatography:

Type	Particulars	Quantity
Glassware	Clean, dry and grease-free glass slide Petri dish Beaker Glass rod	2 units 2 units 3 units 1 units
Chemicals	Adsorbent powder Chloroform Methanol Toluene Acetone	~5 gm 8.3 ml 1 ml 5 ml 4.1 ml
Miscellaneous	Distilled water Hot air oven	30 ml 1 unit

Method:

Preparation:

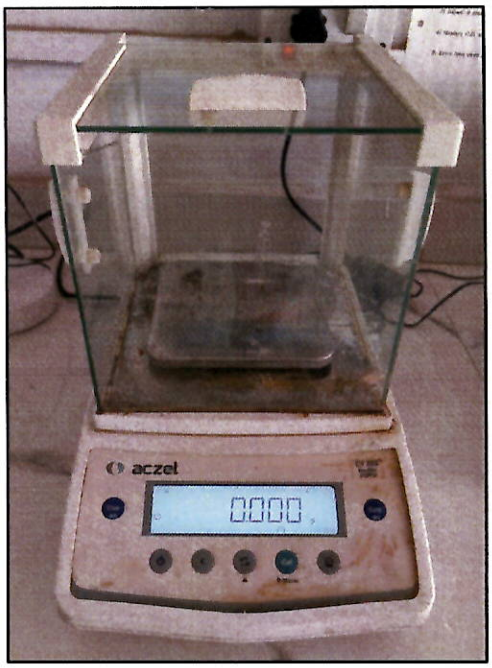
Create adsorbent slurry by mixing an appropriate amount of adsorbent powder with distilled water in a beaker. Pour the slurry on a clean and dry glass slide placed on a petri plate. Let the slurry solidify to form the stationary phase using a hot air oven. Prepare solvent A to run TLC by adding 5 ml chloroform to 1 ml methanol. Prepare solvent B to develop colour in TLC by adding together 5 ml toluene, 3.3 ml chloroform and 4.1 ml acetone.

Running:

Load the plant extracts on the designated spots on the solidified stationary phase. Place the slide into the beaker containing solvent A and keep the beaker covered. Let the liquid phase run upto $3/4^{th}$ length of the slide. Take out the slide and let it dry. Subject it to solvent B in the same manner until colouration develops. Take out the slide and calculate the retention factor for each plant extract.

Instrumentation

Weighing Balance:

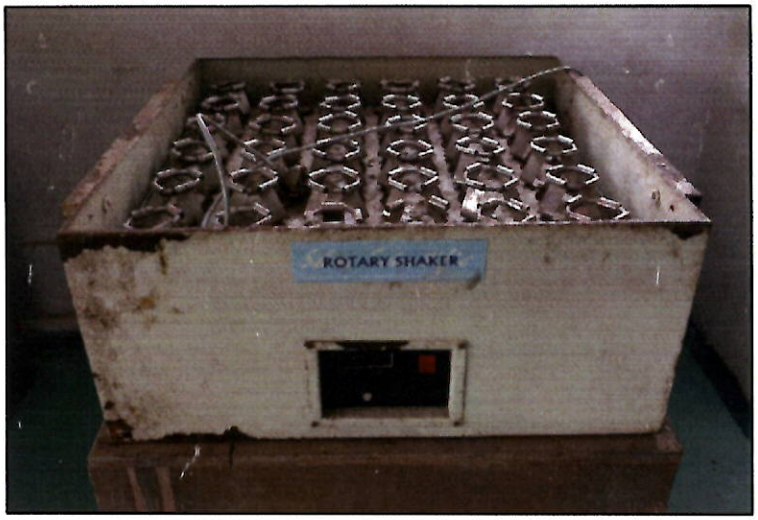


When the object is placed on the scale, the pressure is applied to the sensor, which is an elastic deformation, so that the impedance changes. At the same time, the excitation voltage changes, outputting a change of analog signal. The signal is amplified by the amplifier circuit and output to the analog-to-digital converter, which is converted into a convenient digital signal and output to the CPU operation control. The CPU outputs this result to the display.

Microwave oven:

Microwave ovens work on the principle of conversion of electromagnetic energy into thermal energy. Electromagnetic (EM) energy refers to the radiation (waves) comprising an electrical field and magnetic field oscillating perpendicular to each other. When a polar molecule, i.e., a molecule containing opposite charges, falls in the path of these EM radiations, it oscillates to align with them. This causes the energy to be lost from the dipole by molecular friction and collision, resulting in heating.

Rotary shaker:



A rotary shaker is mainly an LC circuit consisting of a capacitor and an inductor, which produces free oscillations through the interconversion of the electric and magnetic field energy. As the positive feedback becomes stronger and stronger, it leads to a transient steady state. During the transient state, another transistor is gradually charged by the capacitor and then turns on or off, and the state is flipped and another transient state is reached. This way the principle of oscillation is formed over and over again.

Centrifuge:

The centrifuge utilizes the sedimentation principle due to gravitational force. The centrifugation technique uses a centrifugal field to separate particles suspended in a liquid medium. These are put in the centrifuge's rotor either in bottles or tubes. Sedimentation is a process whereby gravity causes suspended particles to separate from fluids. The suspended substance may consist of powder or clay-like particles.

Bacteriological Incubator:

Incubators work on the principle of thermo-electricity. It has a thermostat that maintains a constant temperature by creating a thermal gradient. When any conductor, an electrically-controlled switch used for switching an electrical power circuit subjected to a thermal gradient, generates voltage called a thermoelectric effect. As power is supplied to the circuit predetermined temperature (37°C) is set in the incubator. This temperature is maintained by the compatibility operation of the temperature sensor, the temperature controller and the temperature contactor are major components.

Autoclave:

Autoclaves use pressurized steam as their sterilization agent. The basic concept of an autoclave is to have each item sterilized -whether it is a liquid, plastic ware, or glassware- come in direct contact with steam at a specific temperature and pressure for a specific amount of time. Time, steam, temperature, and pressure are the four main parameters required for a successful sterilization using an autoclave. The most common temperatures used are 121 C and 132 C.

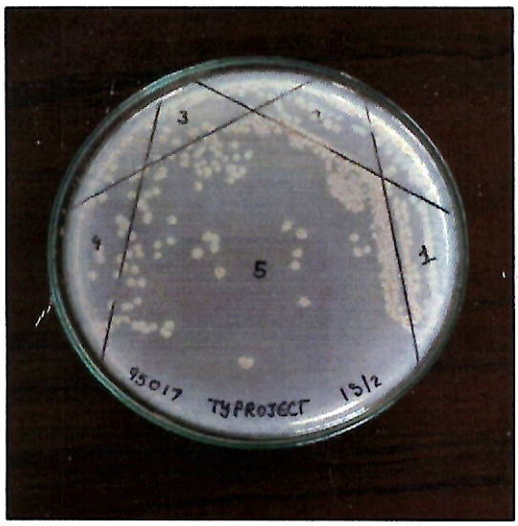
Compound microscope:

Compound microscopes have a mixture of lenses that improves both magnification and resolution. Typically, the specimen or item to be examined is put on a transparent glass slide and positioned between the condenser lens and objective lens on the specimen stage. A condenser lens concentrates visible light from the base onto the specimen.The objective lens collects the light transmitted by the specimen and produces a magnified image of the specimen, known as the primary image, within the body tube. This image is once again amplified by the eyepiece or ocular lens. When higher magnification is necessary, the nose piece is rotated to align the objective of a higher magnification (typically 45X) with the lit portion of the slide. Occasionally it requires very high magnification (e.g. for observing bacterial cell). In this instance, a 100X oil immersion objective lens is utilised.

Observations

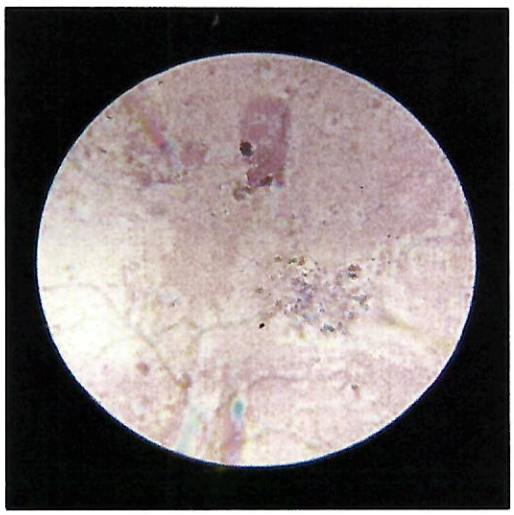
Colony Characteristics:

After skin microflora was inoculated on a Sterile nutrient agar plate and incubated at 37^0C for 24 hours:



Skin Microflora after Incubation for 24 hours at 37^0C

After performing gram staining of the isolated skin microfloral colony, Gram positive cocci were observed under 100x lens of a compound microscope:



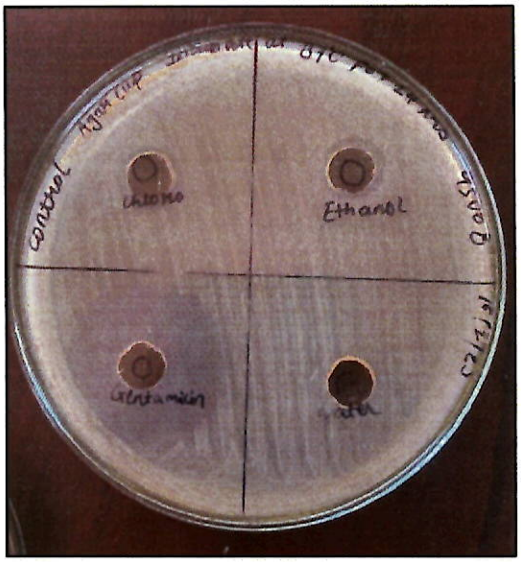
Under 100x lens of a Compound Microscope

Colony characteristics were observed and tabulated as follows:

Size	Shape	Colour	Margin	Elevation	Texture	Opacity	Gram's Nature
0.3 cm	Circular	White	Entire	Convex	Smooth	Translucent	Positive Cocci

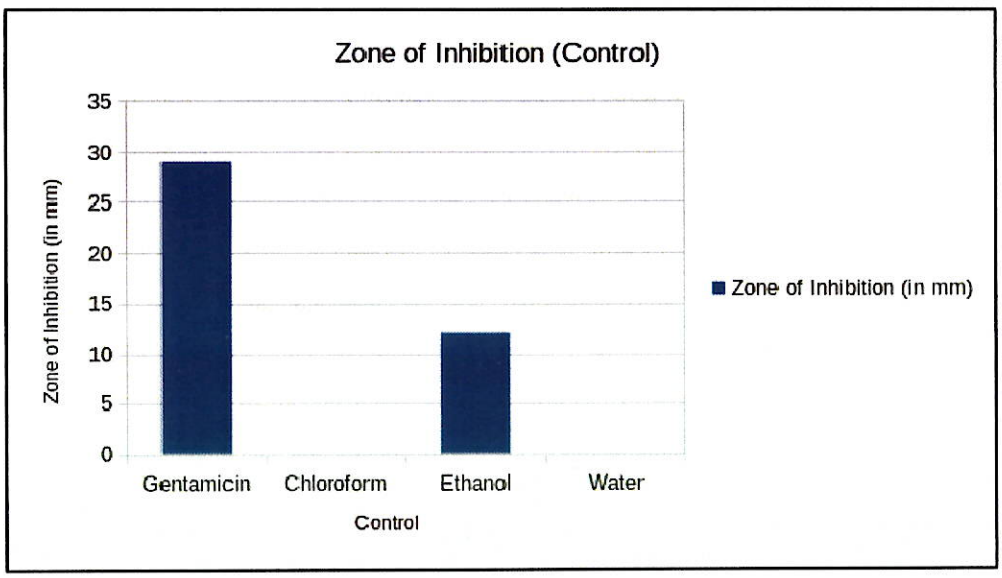
Zone of Inhibition (in mm):

After the Control agar cup plate was incubated at 37^0C for 24hrs:



Control Plate After Incubation

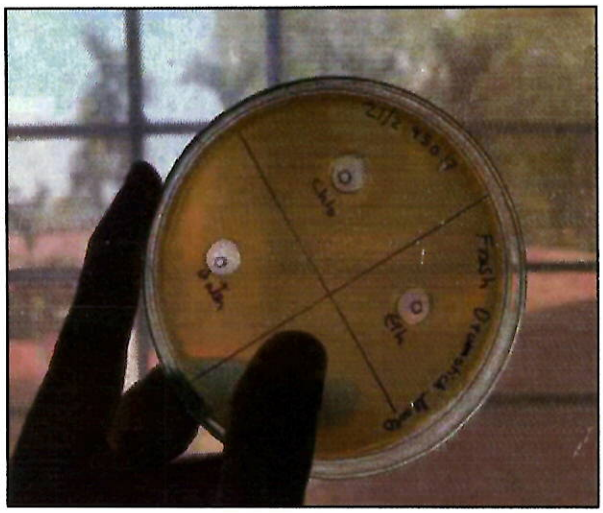
The zones of inhibitions of the Control plate were measured and a bar graph was prepared as follows:



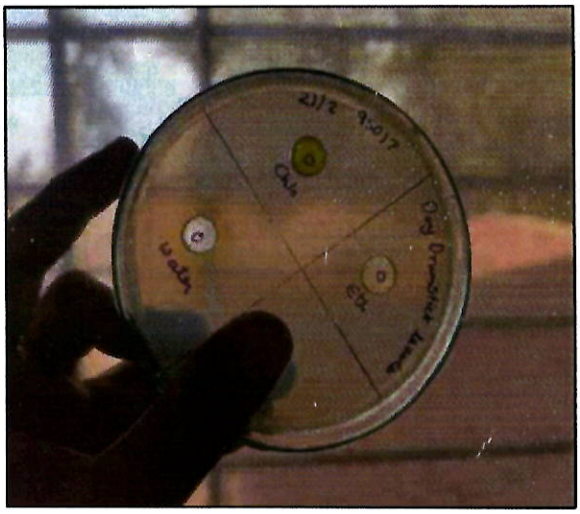
The zones of inhibitions of the Control plate were measured and tabulated as follows:

Control	Gentamicin	Chloroform	Ethanol	Water
Zone of inhibition (in mm)	29 mm	No zone observed	12 mm	No zone observed

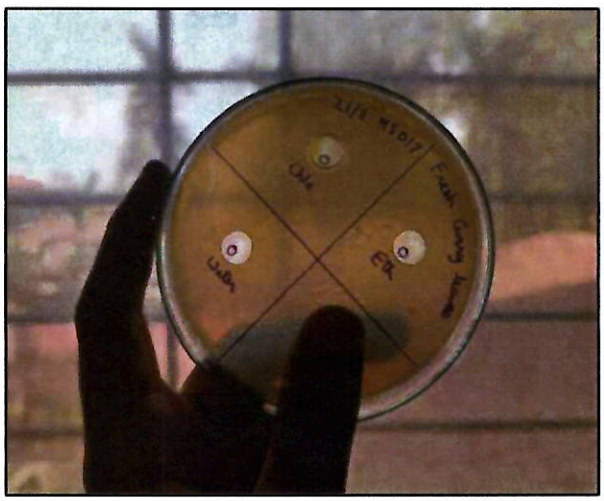
After agar cup plates were incubated at 37^0C for 24 hours:



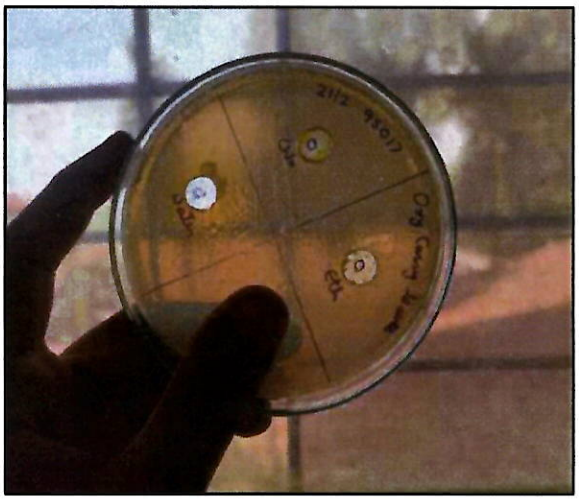
Fresh Drumstick leaf extracts



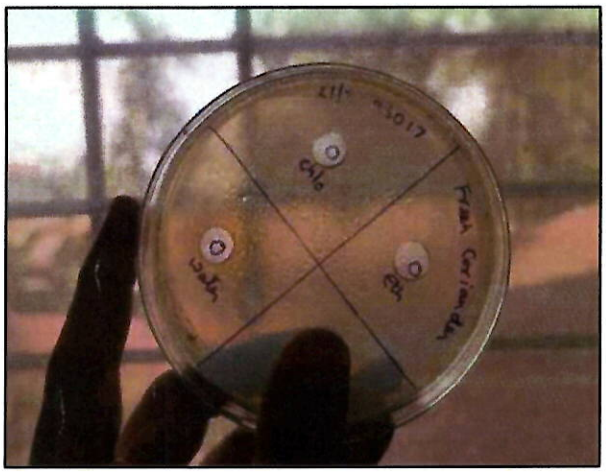
Dry Drumstick leaf extracts



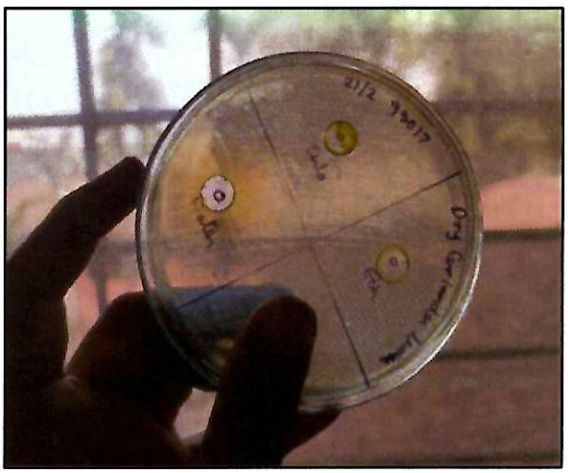
Fresh Curry leaf extracts



Dry Curry leaf extracts

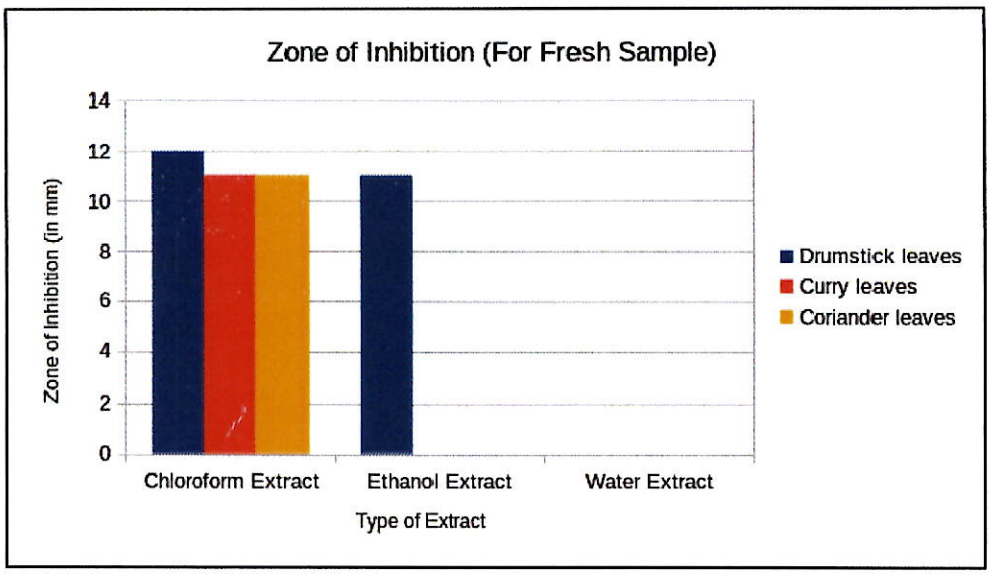


Fresh Coriander leaf extracts



Dry Coriander leaf extracts

The zones of inhibition of each plate were measured and a bar graph was prepared as follows:



The zones of inhibition of each plate were measured and tabulated as follows:

Sample	Type of Sample	Type of extract		
		Chloroform	Ethanol	Water
Drumstick leaves	Fresh	12 mm	11 mm	No zone observed
	Dry	No zone observed	No zone observed	No zone observed
Curry leaves	Fresh	11 mm	No zone observed	No zone observed
	Dry	No zone observed	No zone observed	No zone observed
Coriander leaves	Fresh	11 mm	No zone observed	No zone observed
	Dry	No zone observed	No zone observed	No zone observed

Test for Phytochemicals:

Phytochemical tests for detection of Alkaloids were performed:

Fresh

Dry

Drumstick Leaf Extract

Fresh

Dry

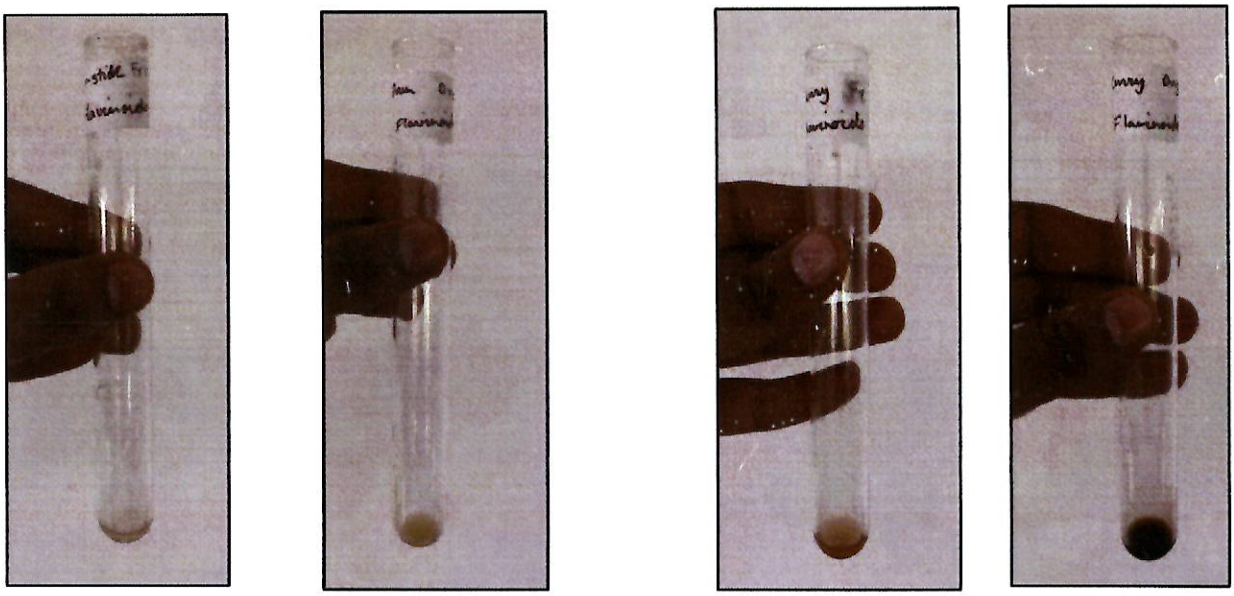
Curry Leaf Extract

Fresh

Dry

Coriander Leaf Extract

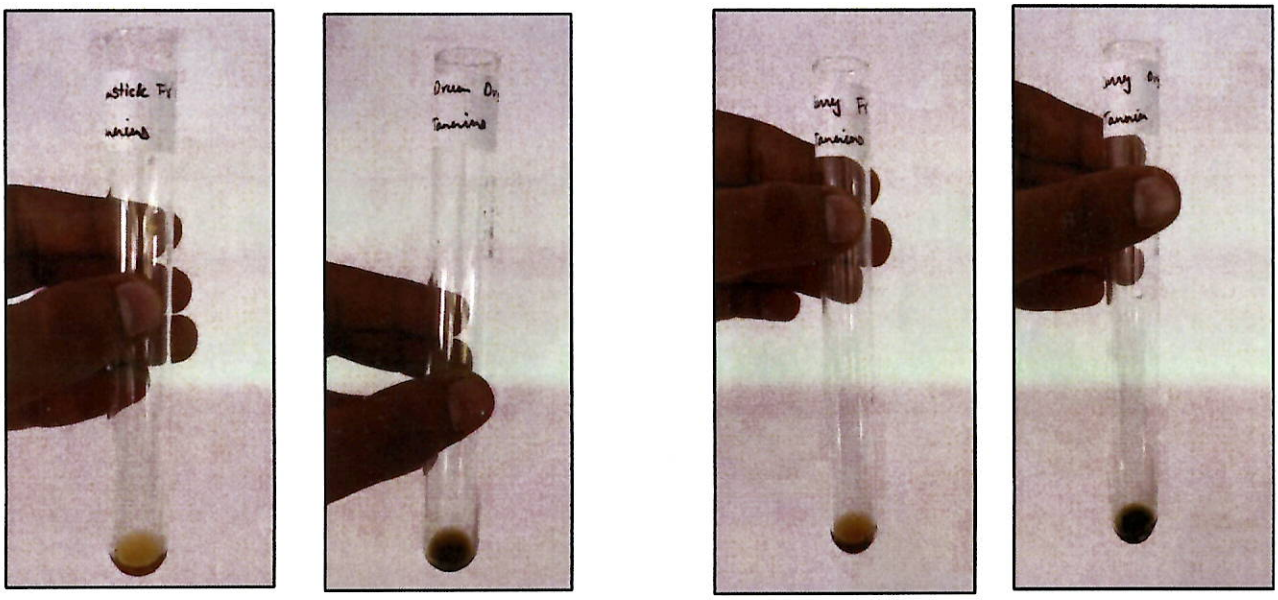
Phytochemical tests for detection of Flavonoids were performed:



Fresh **Dry** **Fresh** **Dry**

Drumstick Leaf Extract **Curry Leaf Extract**

Phytochemical tests for detection of Tannins were performed:



Fresh **Dry** **Fresh** **Dry**

Drumstick Leaf Extract **Curry Leaf Extract**

Phytochemical tests for detection of Phytosterols were performed:

Fresh **Dry**

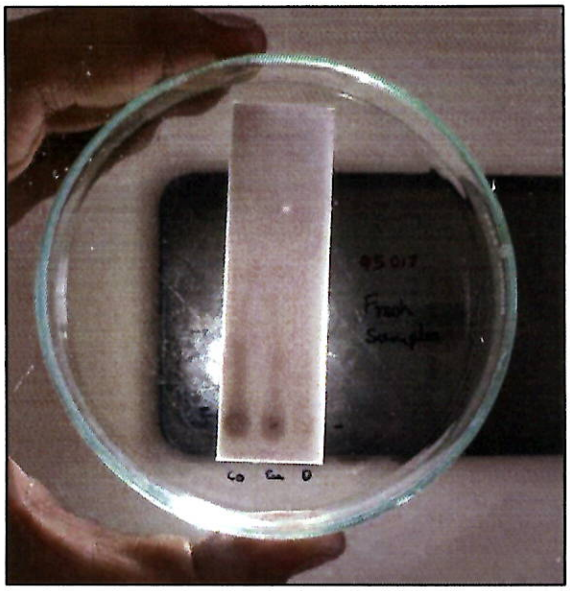
Coriander Leaf Extract

The Presence/Absence of Phytochemicals were tabulated as follows:

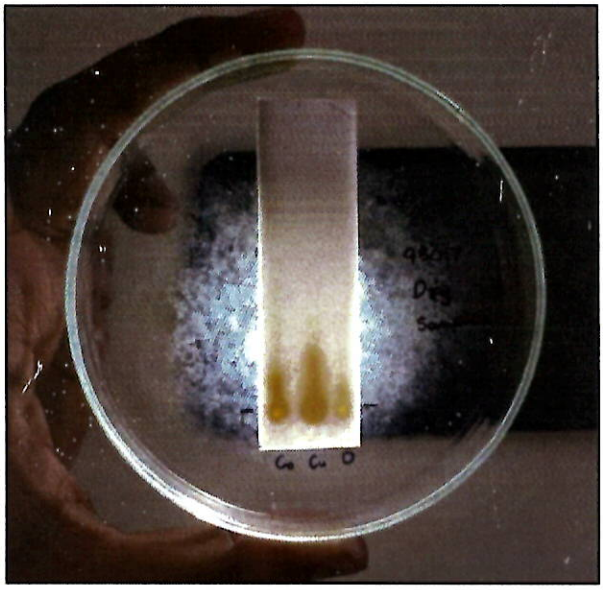
Sample	Type of Sample	Presence of Phytochemicals (+/-)			
		Alkaloids	**Flavonoids**	**Tannins**	**Phytosterols**
Drumstick leaves	Fresh	+ve	-ve	-ve	NA
	Dry	+ve	-ve	+ve	NA
Curry leaves	Fresh	+ve	-ve	-ve	NA
	Dry	+ve	+ve	+ve	NA
Coriander leaves	Fresh	+ve	NA	NA	-ve
	Dry	+ve	NA	NA	+ve

Retention Factor:

Thin layer chromatography was performed:



For Fresh Samples



For Dry Samples

Key:- Co = Coriander leaf extract
Cu = Curry leaf extract
D = Drumstick leaf extract

The Distance travelled by the solutes and the solvent were measured. Retention Factor for each leaf extract was calculated and tabulated as follows:

Sample	Type of Sample	Distance Travelled by Solute (in cm) (x)	Distance Travelled by Solvent (in cm) (y)	Retention Factor (R_f) (x/y)
Drumstick leaves	Fresh	1.4	4.4	0.318
	Dry	1.1	4.6	0.239
Curry leaves	Fresh	1.8	4.4	0.409
	Dry	1.3	4.6	0.282
Coriander leaves	Fresh	1.9	4.4	0.431
	Dry	0.9	4.6	0.195

Results and Discussion

The skin microflora was isolated and identified to be a Gram Positive Cocci.

Now, according to [Collin M. Timm et al. (2020)] : *Staphylococcus* and *Micrococcus* are the most prevalent isolates from skin.

Both of which are Gram Positive Cocci. Due to absence of further IMViC or Sugar fermentation tests we can assume that our isolated skin microbe is of **either *Staphylococcus* or *Micrococcus* species.**

The Microbial Activity of various leaf extracts was studied using agar cup method.

In Drumstick leaf extracts, only two of the fresh sample extracts showed a zone of inhibition, namely the chloroform and the ethanol extracts measuring **12 mm** and **11 mm** respectively. No zone of inhibition was seen in the case of the water extract. None of the dry sample extracts showed a zone of inhibition.

In Curry leaf extracts, only one of the fresh sample extract showed a zone of inhibition, namely the chloroform extract measuring **11 mm**. No zone of inhibition was seen in the case of the ethanol and water extract. None of the dry sample extracts showed a zone of inhibition.

In Coriander leaf extracts, only one of the fresh sample extract showed a zone of inhibition, namely the chloroform extract measuring **11 mm**. No zone of inhibition was seen in the case of the ethanol and water extract. None of the dry sample extracts showed a zone of inhibition.

Various chemical assays were employed to test the plant samples for the presence of phytochemicals.

In Drumstick leaf extracts, the fresh sample extract showed the presence of **Alkaloids** but Flavonoids and Tannins were absent. The dry sample extract showed the presence of **Alkaloids** and **Tannins** but Flavonoids were absent.

In comparison, according to [Piyush Kashyap et al. (2022)] All three phytochemicals are present.

In Curry leaf extracts, the fresh sample extract showed the presence of **Alkaloids** but Flavonoids and Tannins were absent. The dry sample extract showed the presence of **Alkaloids**, **Tannins** and **Flavonoids**.

In comparison, according to [Dr.Rupali Rajvanshi et al. (2019)]. Alkaloids and Tannins are present.

In Coriander leaf extracts, the fresh sample extract showed the presence of **Alkaloids** but Phytosterols were absent. The dry sample extract showed the presence of both **Alkaloids and Phytosterols**.

In comparison, according to [Shreyasi Mallik et al. (2020)]. Alkaloids are present.

Thin layer chromatography of plant water extracts was performed. The distance travelled by the solute and solvent was measured and retention factor for each leaf sample was calculated.

In Drumstick leaf extracts, the fresh and dry sample extracts have the retention factor of **0.318** and **0.239** respectively.

In Curry leaf extracts, the fresh and dry sample extracts have the retention factor of **0.409** and **0.282** respectively.

In Coriander leaf extracts, the fresh and dry sample extracts have the retention factor of **0.431** and **0.195**

Conclusion

The analysis of microbial activity of various extracts of drumstick leaves, curry leaves and coriander leaves was done using agar cup method. The measures of the zones of inhibitions indicate that the chloroform and ethanol extracts of drumstick leaves show better antimicrobial activity than the chloroform and ethanol extracts of curry leaves and coriander leaves on the isolated skin microflora that may be *Micrococcus* or *Staphylococcus* species. The zones of inhibitions also indicate that chloroform is a better solvent for extracting phytochemicals form all three samples. The phytochemical tests indicate that alkaloids were present in both fresh and dry forms of all three samples and if compared to the pattern indicated by the zones of inhibitions, they may be responsible for the chief antimicrobial activity. Thin layer chromatography indicates that fresh extracts of all three samples had a higher solubility in the mobile phase as compared to their dry counterparts.

Future Prospects

- To identify the skin microflora used.
- To identify the phytochemicals by techniques such as FTIR.
- To test and approve obscure vedic knowledge by conducting similar research on other plants and herbs.

References

Articles and research papers:

1] B. Mylarappa, B. L Ningappa, R. Dhananjaya, R. Dinesh, Harsha, and L. Srinivas, Food Chemistry. 118, 747-750 (2010).

2] Dr.Rupali Rajvanshi1, Dr.Kusum Mittal; Phytochemical Analysis of Curry Leaves; 367; Volume 8 Issue 9, September 2019.

3] Dr.Vanisha S. Nambiar, NUTRITIONAL POTENTIAL OF DRUMSTICK LEAVES: AN INDIAN PERSPECTIVE-text January 2023, page no 2.

4] Junaid R Shaikh and MK Patil Qualitative tests for preliminary phytochemical screening: An overview.

5] Kedarnath1, KambleKaveri M2, Vishwanath B, Chimkod and C S Patil3 on Antimicrobial Activity of aloe vera leaf extract in International Journal ofApplied Biology and Pharmaceutical Technology,Volume-4 , Issue-4 , Oct-Dec 2013.

6] NanditaKamat, Diana Pearline, and Padma Thiagarajan on Murrayakoenigii (L.) (Curry Leaf): A Traditional Indian Plant, Research Journal of Pharmaceutical, Biological and Chemical Science, September – October 2015 RJPBCS 6(5) Page No. 691.

7] PiyushKashyap Et al. Recent Advances in Drumstick (Moringaoleifera) Leaves Bioactive Compounds: Composition, Health Benefits, Bioaccessibility, and Dietary Applications, *Antioxidants* 2022, *11*(2), 402.

8] Saeed. Antimicrobial activities of emblicaofficinalis and Coriandrumsativum against gram positive bacteria and Candida albicans. Pak. J. Bot. 2007;39(3):913-917.

9] ShreyasiMallik Et al. Phytochemicals of Coriander, Cumin, Fenugreek, Fennel and Black Cumin: A Preliminary Study; Volume 45; 478.

10] Ugochukwu S.C., Arukwe U.I., Onuoha I.; Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetiatripetala G. Baker. Asian J. Plant Sci. Res; 3(3):10-13,(2013).

11] Zare-Shehneh. Biological activities of a new antimicrobial peptide from Coriandrumsativum. Int J Biosci. 2014;4(6):89-99

Links: (Accessed within 18th to 28th March, 2023)

1] https://www.locosc.com/What-s-the-Working-Principle-of-Digital-Electronic-Weighing-id3231464.html

2] https://studiousguy.com/microwave-oven-working-principle/

3] https://antiteck.com/rotary-shaker-2/

4] https://microbenotes.com/centrifuge-principle-parts-types-uses-examples/

5] https://medicallabnotes.com/incubator-introduction-principle/

6] https://pharmawiki.in/autoclave-sterilization-principle-working-pdf-ppt-autoclave-validation-autoclave-diagram/

7] https://microbiologynote.com/compound-microscope-principle-parts-diagram-definition-application/

ISOLATION, IDENTIFICATION OF RHIZOBIUM FROM ROOT NODULES OF FENUGREEK PLANTS FROM AREA OF DAHANU (KOSBAD) AND TO STUDY ITS EFFECT ON SOIL FERTILITY AND PLANT GROWTH.

A PROJECT SUBMITTED

TO THE UNIVERSITY OF MUMBAI TOWARDS

PARTIAL FULFILMENT OF THE DEGREE OF

BACHELOR OF SCIENCE IN BIOTECHNOLOGY

UNDER THE GUIDANCE OF

MRS. RIDDHI J. BISHT

(ASSISTANT PROFESSOR OF BIOTECHNOLOGY DEPARTMENT)

SUBMITTED BY:-**MISS. PALAK MAHESH BAJPAI**

DEPARTMENT OF BIOTECHNOLOGY

SDSM COLLEGE

PALGHAR

T.Y.B.Sc BIOTECHNOLOGY

(2022-23)

Sonopant Dandekar Shikshan Mandali's
SONOPANT DANDEKAR ARTS, V. S. APTE COMMERCE AND M. H. MEHTA SCIENCE COLLEGE
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website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com
(NAAC Reaccredated 'B' Grade)

Ref. No.: Date : 31/03/2023

CERTIFICATE

The work described in this project entitled "ISOLATION, IDENTIFICATION OF RHIZOBIUM FROM ROOT NODULES OF FENUGREEK PLANTS FROM AREA OF DAHANU (KOSBAD) AND TO STUDY ITS EFFECT ON SOIL FERTILITY AND PLANT GROWTH" has been carried out independently by MISS. PALAK MAHESH BAJPAI Exam Seat no. 4015383 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

31/3/23.

Signature of Guide

Signature of H.O.D

Signature of Principal

Head of the
Biotechnology Department
S. D. S. M. College,
Palghar (W) - 401 404

5/4/23

Signature of Examiner

DANDEKAR ARTS, APTE COMMERCE, MEHTA SCIENCE COLLEGE
PALGHAR
DIST. THANE

DECLARATION

I, **PALAK MAHESH BAJPAI** Student of T. Y. B.Sc. Biotechnology, hereby declare that the project entitled "Isolation, Identification of Rhizobium from Root Nodules of Fenugreek plants collected from area of Dahanu (Kosbad) and to study its effect on soil fertility and plant growth." submitted by me for the academic year 2022-2023, is based on the actual work carried out by me under the guidance of my professor **MRS. RIDDHI J. BISHT**. I further state that this work is original and no part has been presented for any degree, diploma or similar title of any university.

ACKNOWLEDGEMENT

I am highly indebted and express my deepest sense of gratitude to my guide **Prof. Riddhi J. Bisht** and Head of the department, Biotechnology **Dr. Shilpa Gharat** for their valuable guidance and suggestions throughout the research work and preparation of this project.

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I would like to expresses gratitude to the Principal and management of Sonopant Dandekar Arts, V.S.Apte commerce and M.H.Mehta science college, Palghar for permitting to conduct the project work in the institute

. I would also like to thank other professors, **Prof. Shailaja Palan, Prof. Runali Raut, Prof. Apurva Save, Prof. Ishwari Mehta, Prof. Niyati Patil, Prof. Lisa Sam, Prof. Hardik Churi, Prof. Sajari Raut**. Lab Assistant **Jidhnaya Patil** and Lab Attendant **Nitin Jadhav, Nitesh Pagi, Bhavesh Bhoir and Bhupendra Raut, Suvidha Jadhav** for their immense guidance and concern throughout my project work and helping wherever required.

Finally, I wish to extend a warm thanks to everybody involved directly and indirectly with my work.

TABLE OF CONTENTS

ABSTRACT

Nowadays, the population has taken a great rise and this has directly been showing the high demand for food. As the population is increasing the demand for food is increasing and this is directly impacting the stress to the agriculture. As the demand of food has taken rise the cultivation of crops for consumption is gradually increasing and for increasing the productivity of crops the farmers use chemical fertilizers for the growth of crops. The chemical fertilizer use has shown increase in various types of pollution which is slowly harming the environment. To avoid such various pollution alternative way is to use biofertilizers, this will help to prevent environmental damage and the pollution caused will be avoided. Among this one of the known biofertilizers that can be used is Rhizobium which is symbiotic nitrogen fixing bacteria. In this study, the isolation and identification of Rhizobium is done by using the isolation technique and confirming it by the biochemical tests. The further study was continued by inoculating the isolated and identified culture in the soil, were the nitrate content of the soil for different samples was determined by the colorimetric method and also by checking the effect of nitrate content on the plant growth by measuring the height of it. It was observed that rhizobium shows an increase in soil nitrate content which directly is observed by the growth of plant. So use of chemical fertilizers shows adverse effect on the environment which can be suppressed by using this alternative that is use of biofertilizers.

Keywords: Rhizobium, Isolation, Biofertilizers, Nitrate content, Symbiotic Nitrogen fixing bacteria

INTRODUCTION

In the current scenario most of the agricultural lands are been deprived of minerals, which is one of the main component which is essential for the plant growth. The more use of chemical fertilizers have made the soil and atmosphere very polluting and harmful as the excess pollution harms the human, animals and plants life the same way the excess use of chemical based fertilizers is harming the soil microorganisms and makes the soil conditions harsh for suitable growth of plants. In the increase in population the food demand has also taken a great height and this rise in population is directly affecting the agricultural fields. For large scale productivity of crops due to rising demand the farmers use chemical fertilizers and this will show adverse effect in future which will be very harmful for the world for survival purpose. The demand of food supplies can also be fulfilled by using the 'Biofertilizers' for the crop production.

'Biofertilizers' are the substances that contain microorganisms which when added to the soil increases its fertility and promotes plant growth. Naturally few microorganisms present in soil helps fertility of soil which helps in plant growth organisms such as Rhizobium is soil habitat bacteria which helps to colonize with legume root and show fixation of atmospheric nitrogen symbiotically which is seen in the form of root nodules. As the farmers farming and crop production concerns, they have high expense of cost for buying the chemical fertilizers which sometimes cost effective to then using biofertilizers and even using a technique that planting such legume plants which generally are one of the best known way of nitrogen fixers and helps in the better crop yields. Such crops can planted around the other plants and help to increase in the nitrogen fixation of the atmosphere by utilizing it. The one of other main reason of this research is to let the upcoming agricultural field workers get more knowledge about the relation of the legume plants and the nitrogen fixing activity so that the many different positive possibilities can be achieved such as high yield of crops will help in marketing level for demand of biofertilizer for betterment of crops and the environmental conditions will be under control which is beneficial for future generations. Addition of biofertilizers will help the soil to be fertile and healthy for plants growth. It will fix other micro nutrients like Nitrogen, Phosphorus, Sulphur, etc. Biofertilizers can be easily applied in the soil. Isolation of the bacteria is being done to identify the infecting bacterial strain with infects the root of legumes and induces the formation of nodules. The Rhizobium bacteria used as biofertilizers which are used in cultivation of crops can be applied in some ways that is by sprinkling biofertilizer in the water bed for a period of time and then the seeds are be sown, then it can also been added by dipping the seeds into the mixture of Nitrogen and Phosphorus fertilizers and dry it and then sown also as known it can be added directly in the soil where the seeds have to be sown. To isolate the Rhizobium bacteria CRYEMA is been used because Yeast extract serves as a good source of readily available amino acids, vitamin B complex and accessory growth factors for Rhizobia. It also poises the oxidation-reduction potential of medium in the range favourable for Rhizobia and serves as hydrogen donor in respiratory process. Mannitol is the fermentable sugar alcohol source. Magnesium provides cations essential for the growth of Rhizobia. Congo red inhibits penicillin-susceptible strains.

Root Nodules

Applications:-

1. The Rhizobium biofertilizers is applied to plants surfaces, seeds or in the soil where the bacteria will colonize the rhizosphere or interior of plant and promotes plant growth.
2. Biofertilizers helps in increase of crop yield.
3. Improves and restores soil fertility.
4. Reduces the production cost and less harm from chemical fertilization.

RATIONALE

Plant materials remain as an important resource and the Rhizobium is the bacteria that live in symbiotic association with the Root nodules of leguminous plants. Fixation of Nitrogen cannot be done independently. Rhizobium is a vital source in soil. They fix nitrogen and convert the nitrogen into ammonia. Agricultural Nitrogen fixation helps in increasing soil productivity and soil fertility. Rhizobium is a biofertilizer, biofertilizers are substances that contain micro-organisms, which when applied to the soil, it increases the nutrient content and enhances the plant growth. The urge to protect environment from various types of pollution which is also produce in some amount from overall pollution is by chemical fertilizers which are been used in agricultural fields for the cultivation of crops. And one of such effective and less cost consuming way is to use biofertilizers. As the nitrogen fixation helps in increasing the soil fertility and better yield of crops. The use of biofertilizers will be cost friendly to farmers and they will get better crop yield and will give a rise in the yield of crops by natural method.

REVIEW OF LITERATURE

There is a huge necessity of biofertilizers for the better crop yield and is less cost effective, affordable for the farmers. By using biofertilizers crop diseases are been reduced and also helps pollution free environment which will be helpful for the future. Addition of biofertilizers will help the soil to be fertile and healthy for plants growth. It will fix other micro nutrients like Nitrogen, Phosphorus, Sulphur, etc. Biofertilizers can be easily applied in the soil. Isolation of the bacteria is being done to identify the infecting bacterial strain with infects the root of legumes and induces the formation of nodules.

The isolation of bacteria, effect of nitrate content of soil and plant growth was studied by referring research paper of several authors :-

- F. Shahzad*, M. Shafee, F. Abbas, S. Babar, M. M. Tariq, and Z. Ahmad, Isolation and biochemical characterization of Rhizobium meliloti from root nodules of alfalfa (Medico Sativa). The Journal of Animal and Plant Sciences, 22(2): 2012, Page: 522-524

  This review emphasizes on isolating the bacteria from the nodules of legume plant. The author also characterizes the bacteria by specific biochemical test for proper isolating process of the bacteria. Overall here author has explain in a very short and easy way the process has be covered and specification has been mentioned throughout.

- Gauri, Singh AK, Bhatt RP, Pant Shailja, Bedi (2011): Characterization of Rhizobium isolated from root nodules of *Trifolium alexandrinum*. J Agric Technol 7(6):1705-23

  Here author draws attention to the isolation of Rhizobium bacteria from the clover crops. The characterization helps in the specification of the bacteria which can be further utilize for other various purposes in future prospects. The main aim is to isolate the bacteria from the crop and to have a conformational idea of presence of bacteria in the sample which is done in a very good manner with all the possible correct methods.

- PawarVaishali A., Pooja R. Pawar, Ashok M. Bhosale and Sourabh V. Chavan (2014): Effect of Rhizobium on Seed Germination and Growth of Plants, Journal of Academia and Industrial Research, Volume 3, Issue 2, July 2014.

  The review of this research helps to understand the germination process of the seeds when in presence of Rhizobium bacteria which shows the clear idea of how the bacteria is helpful in the soil fertility and the growth of plant. It also emphasizes on using it as the biofertilizers which very helpful for the plant and soils fertility.
  The author has made the concept clear of using the bacteria as a biofertilizer.

AIM AND OBJECTIVE

Aim:- To isolate and identify the Rhizobium bacteria from Root Nodules of Fenugreek plants from various two localities of Dahanu (Kosbad) and to check its effect on soil fertility and plant growth.

Objectives:-

1. To isolate whether the organism from the root nodules of Fenugreek plants.
2. To perform Biochemical test to check the bacteria isolated is able to utilize sugars by sugar fermentation test and others test which are included in the Biochemicals.
3. To check the effect of Nitrate content of soil which is helpful for better crop yield by taking nitrogen from atmosphere and converting it into ammonia.
4. To observe the plant growth and comparing the bacterial activity with the standard.

REQUIREMENTS

1 Surface Sterilization:-

Type	Particular	Quantity
Sample	Root Nodules of Fenugreek plants from Sample 1[Area 1] and Sample 2 [Area 2]	6 to 7 nodules each
Chemicals	1] 0.1% Mercuric Chloride (HgCl2) 2] 20% Tween 20 Detergent	10ml 10ml
Glassware	1] Grease free glass slide 2] Sterile Petri plate	2 per each sample 2 per each
Miscellaneous	1] Sterile Distilled water 2] 75% Ethanol 3] Sterile Forcep 4] Weighing Balance 5] Stored at 4°C	50ml 1 unit 1 unit 1 unit

2 Isolation of Nitrogen Fixing Bacteria (Rhizobium):-

Type	Particular	Quantity
Sample	Rhizobium Suspension Sample 1 Sample 2	
Media	Sterile Congo Red Yeast Extract Mannitol Agar	100ml
Glassware	1] Sterile Petriplate 2] Sterile Pipette 3] Conical flask	4 unit 1ml-2 250ml-1
Miscellaneous	1] Tripod stand 2] Nichrome loop 3] Incubate at 37°C for 2-3 days	1 unit 1 unit 1 unit

3 Morphological Characteristics:-

Type	Particular	Quantity
Sample	White Translucent colonies picked from CRYEMA plate	
Reagent	1] Crystal Violet 2] Grams Iodine 3] 95% Alcohol 4] Saffranine	
Glassware	Grease Free Slide	2 per each
Miscellaneous	1] Cedarwood oil 2] Microscope (Oil Immersion 100 x lens) 3] Dropper 4] Nichrome loop	 1 unit 1 unit 1 unit

4 Biochemical Tests:-

Type	Particular	Quantity
Sample	18hrs old culture of Rhizobium for Sample 1 Sample 2	
Sugar Fermentation Tests	1] Sterile 1% Glucose with Inverted Durhams tube and Andrades Indicator.	2 units
	2] Sterile 1% Lactose with Inverted Durhams tube and Andrades Indicator.	2 units
	3] Sterile 1% Mannitol with Inverted Durhams tube and Andrades Indicator.	2 units
	4] Sterile 1% Maltose with Inverted Durhams tube and Andrades Indicator.	2 units
IMVIC Tests	1] Indole Test- 2% Tryptone water Reagent: 2 to 4 drops of Kovac's reagent	2 units
	2] Methyl red test- 1% Glucose Phosphate Broth Reagent: 5 drops of Methyl Red reagent	2 units
	3] Vogus Proskauer Test- 1% Glucose Phosphate Broth Reagent: 4 drops of Omera's reagent (α-Napthyl + 40% KOH)	2 units
	4] Simmon Citrate Agar Test- Sterile Simmon Citrate Agar slant	2 units
Other Tests:-	1] Triple Sugar Ion Test:- Sterile TSI slant with loopful of culture	2 units
	2] Urease Test:- Sterile Christensen's Urea broth with loopful of culture	2 units
	3] Catalase Test:- 2 drops of Hydrogen peroxide- one colony picked	2 units
	4] Starch Hydrolysis Test:- Sterile 1% Starch Agar plate Reagent: Lugol's Iodine (Few drops)	2 units
	5] Glucose Peptone Agar Test:- Sterile Glucose Peptone Agar plate	2 units
Glassware	1] Sterile Test tubes with inverted Durham's tube	8 units
	2] Sterile Test tubes	12 units
	3] Sterile Petri plate	4 units
	4] Grease free cavity slide	2 units
	5] Dropper	1 unit
Miscellaneous	1] Test tube stand	2 units
	2] Incubate at 37% for 24 hours	1 unit
	3] Nichrome loop	1 unit
	4] Nichrome loop (butt	1 unit

4 Effect of Rhizobium on soil nitrate content and plant growth:-

Type	Particular	Quantity
Sample	Test:- 10ml of Rhizobium culture + 100ml of Distilled water for both samples Control:- Distilled water [100ml]	
Reagents	1] Zinc dust (one pinch) 2] 0.5ml of NEDD [N-(1-Naphthyl) ethylenediamine] 3] 0.5ml of Sulphanilamide reagent	 50ml 50ml
Glassware	1] Test tubes 2] Pipettes 3] Conical flask	15 units 1ml-4 units 10ml- 4 units 4 units
Miscellaneous	1] Measuring cylinder 2] Sterile Soil 3] Colorimeter at 540nm	1 unit 1 unit

For plant growth:-

Type	Particular	Quantity
Sample	10 Healthy Fenugreek seeds	
Reagents	0.1% HgCl2	
Glassware	1] Sterile Petri plate 2] Beaker	2 units 2 units
Miscellaneous	1] Plastic pots 2] Sterile Distilled water 3] Sterile Forcep	4 units 200 ml 1 unit

Media Composition

1] Congo Red Yeast Extract Mannitol Agar [CRYEMA]:-

For 200ml

Media	Amount
CaCO3	0.5gm
MgSO4 7H2O	0.04gm
K2HPO4	0.10gm
Yeast Extract	0.02gm
NaCl	0.02gm
Congo Red (1%)	0.5gm
Agar	6gm

[1% Mannitol => 1% add separately
1gm Mannitol+100ml Distilled water
Autoclave it
Add 25ml in 200ml of CRYEMA]

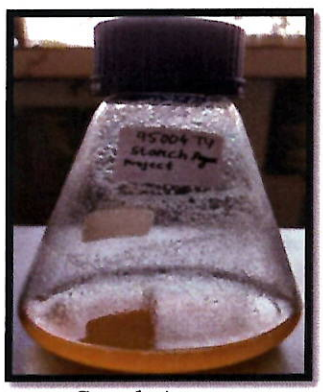
2] For Sugar Fermentation Test:-

1% Glucose	1% Lactose	1% Mannitol	1% Maltose
Glucose- 1gm	Lactose- 1gm	Mannitol- 1gm	Maltose- 1gm
Peptone- 0.5gm	Peptone- 0.5gm	Peptone- 0.5gm	Peptone- 0.5gm
Distilled water- 100ml	Distilled water- 100ml	Distilled water- 100ml	Distilled water- 100ml
Andrade Indicator- 1ml	Andrade Indicator- 1ml	Andrade Indicator- 1ml	Andrade Indicator- 1ml
----------With	Inverted Durhams Tube	----------	----------

3] 1% Glucose Phosphate Broth:-
1] Peptone- 0.25 gm
2] Dextrose- 0.25 gm
3] K2HPO4- 0.25 gm
4] Distilled Water- 100 ml

4] Sterile 1% Starch Agar Plate:-
1] Beef Extract- 0.15 gm
2] Soluble Starch- 0.5 gm
3] Agar- 0.6 gm
4] Distilled Water- 50 ml

5] Sterile Glucose Peptone Agar Plate:-
1] Agar- 1.25 gm
2] Peptone- 0.5 gm
3] Glucose- 2 gm
4] pH- 5.6
5] Distilled Water- 50 ml



Starch Agar

Glucose Peptone Agar

METHODOLOGY

Study Area:-

Kosbad is a small developing area in Palghar district in Maharashtra, India which is one of the known place for doing small scale farming located in Dahanu taluka area. Its Latitude 20°02'37"N and Longitude 72°44'59"E. The sample used in this research paper that is Fenugreek plants, are scientifically known as *Trigonella foenum graecum* having healthy root nodules were been collected from two different areas near the Kosbad village. The sample were been kept in sealed packed bags from the site and kept under cool temperature until used.

Area 1

Area 2

Methods:-

Extraction of Rhizobium bacteria from Root Nodules:-

Initially detached roots of Fenugreek plants were washed under tap water to remove adhere soil from surface of nodules. Then the pink, healthy root nodules were removed carefully by using sterile forceps under sterilized conditions. Then the process for surface sterilization of root nodules were been done by treating it with 20 % Tween 20 Detergent and then washed with Sterile Distilled water. After this, the root nodules were washed with 0.1% Mercuric Chloride [HgCl2] for 2 minutes, then washed with Sterile Distilled water for 3 times. After this the root nodules were washed with 75% ethanol for 2 minutes. Finally, the root nodules were washed with sterile distilled water for 7 times. Nodules were taken on slide and teased with sterile forceps. White exudates from nodules were streaked on sterile CRYEMA plate (Congo Red Yeast Extract Mannitol Agar of pH 6.8±2) by using a flame sterilized nichrome loop. The streaked plates are then incubated at 37°C for 3-4 days. Rhizobium bacteria will form white- coloured colonies, while other organisms will give red colonies. Rhizobium bacteria does not allow Congo red inside its cell and therefore give white colonies. The White translucent colonies developed on the plate and the colony characteristics were observed for size, shape, colour, opacity, elevation, margin and then gram staining was been carried out.

Gram staining of colonies:-

The colonies obtained after incubation was then been selected for gram staining. A smear was made on a grease free microscopic glass slide. Then the smear was treated with 1% Crystal violet for 1 minute. After that wash smear with distilled water. Then, 1% of Gram's Iodine was added and kept for 1 minute. Then the slide was washed with distilled water followed with washing with decolourizer that is 95% Alcohol for 30 seconds. Then the slide was counterstained that is 1% Safranine for 1 minute. Lastly the slide was washed with distilled water. The slide was air-dried. One drop of Cedarwood oil (immersion oil) was added on the smear. The slide was observed under the microscope (100Xlens).The Gram's nature and shape was observed and noted.

Biochemical tests:

1] Sugar fermentation test:

This test is been done to find out which types of sugar is utilized by Rhizobium bacteria, a sugar fermentation test was been carried out. Were loopful of culture was inoculated in sterile test tubes having 1% Glucose, 1% Lactose, 1% Mannitol 1% Maltose. Test tubes were incubated at 37°C for 24 hours. Change in colour of media and gas production in Durham's tube was been observed and results were been noted.

2] IMVIC TEST:

a) Indole test:

To determine whether the bacteria organism has the ability to convert tryptophan into indole due to the presence of enzyme "Tryptophanase", an Indole test was been performed. Loopful of culture was inoculated in sterile 2% Tryptone water and tube was incubated at 37° C for 24 hours. Then 2 to 3 drops of Kovac's reagent was added in inoculated, incubated tubes for both the samples. The Colour of the ring was been observed.

b) Methyl Red test:

Microorganisms present in the samples was been tested by methyl red test, were loopful of culture was been inoculated in sterile 1% Glucose phosphate broth for both samples. The tubes were incubated at 37°C for 24 hours. Then 3 drops of methyl red reagent were added after incubation. A colour change and ring formation was noted.

c) Voges Proskauer Test:

To determine whether an organism can synthesize acetoin or not. Loopful of culture was inoculated in sterile 1% Glucose phosphate broth. The tubes were incubated at 37°C for 24 hours 4 drops of Omera's reagent was added. The result was observed and noted.

d) Simmon Citrate agar Test:

To check whether an organism can use citrate or not. loopful of culture was streaked on the surface of sterile Simmon citrate agar slant and slant was incubated at 37°C for 24 hours. Change in colour was been noticed.

iii) Triple sugar ion test:-

This test was been performed by taking loopful of culture on sterile straight loop and then it was stabbed in sterile TSI slant incubated at 37°C for 24 hours. The change in colour was recorded. This test will help to find out which sugars were utilized by bacteria.

iv) Urease test:

To determine whether the isolated bacteria is Urease producer or not, loopful of culture was inoculated in sterile Christensen's urea broth and incubated at 37°C for 24 hours and change in colour was observed.

v) Catalase test:-

One colony was picked from the two plates and kept on the slide. Then 2 drops of hydrogen peroxide were added on it. The presence or absence of effervesces was been observed. The presence of effervesces indicates an organism is a catalase synthesizing organism or not.

vi) Starch hydrolysis test:-

Sterile 1% starch agar plate was taken. One loopful of culture was streaked on the plates. Both the plates was incubated at 37°C for 24 hours. After incubation, few drops of Lugol's iodine was added on the plates. Change in colour due to the presence or absence of starch was recorded. If the clear zone was observed around a colony, indicate, bacteria can produce an amylase enzyme.

vii) Glucose peptone agar test:
Loopful of culture was streaked on sterile Glucose peptone agar plates and incubated at 37°C for 24 hours. The presence or absence of colonies after incubation was recorded. Rhizobium cannot grow on this media because this organism cannot utilize nutrients available in media.

Effect of rhizobium on soil nitrate content and plant growth:-
Take Sterile soil. Divided it into two equal half. One half was inoculated with 100 ml as control of rhizobium suspension having density 1.5×10^9 cells/ ml while second was uninoculated and same was for the sample 2 also. The density of suspension was been compared and obtained by using MC Farland method by using spectrophotometer at 620nm which helps in obtaining approximate number of bacteria present in the suspension.

1. **To check the nitrate content of soil:**

For both samples 1 and 2, 1gm of sterile soil was taken and it was mixed with 10 ml of water. Then it was mixed properly and was allowed to settle down. After settling the supernatant was transferred to the new test tube. One pinch of zinc dust was added and allowed to stand for a few minutes. Then the supernatant was mixed with 0.5ml of NEDD (N-(1-Naphthyl) ethylenediamine) and 0.5 ml of sulphanilamide reagent. The O.D. was measured by using colorimeter at 540 nm. The same procedure was repeated for both samples uninoculated soil also. Soil nitrate content was measured daily for 10 days. The standard readings was taken by using standard nitrite solution with a concentration of 100 ug/ml. Reading was taken at 540mm using colorimeter. This test is very beneficial to check the nitrate content present in the soil so that it will help in plant growth and less of the chemical fertilizers will be used.

The nitrate content of the soil was calculated using the following formula:-

$$\text{The nitrate content of soil} = \frac{\Delta \text{ O.D. of Sample}}{\Delta \text{ O.D. of Standard}} \times \text{Concentration of Standard} \times \frac{1000}{\text{Volume of Sample}}$$

ii. Plant growth:
For observing the plant growth ten surface-sterilized healthy fenugreek seeds were been added in both inoculated and uninoculated soil for both the samples. Then both the samples of inoculated and uninoculated soil with surface sterilized seeds was kept for incubation in light and dark at room temperature . The plants germination growth and the height was been observed up to 10 days.

INSTRUMENTATION

Weighing Balance-

A weighing balance is a device used to measure weight or mass. These are also known as mass scales, weight scales, mass balance, weight balance or simply balance scale. It displays weight as a number, usually on a liquid crystal display (LCD). They are versatile because they may perform calculations on the measurement and transmit it to other digital devices. An analytical balance is a class of balance designed to measure small mass in the sub-milligram range. The measuring pan of an analytical balance is inside a transparent encloser with doors so that dust does not collect and so any air currents in the room do not affect the balances operation. This enclosure is often called a draft shield, the use of a mechanically vented balance safety enclosure, which has uniquely designed acrylic air foils, allows a smooth turbulence - free air flow that prevents balance fluctuation and the measure of mass down to 1 µg without fluctuations or loss of products. Also, the sample must be at room temperature to prevent natural convection from forming air currents inside the encloser from causing an error in reading.

The basis of the rapid & exact working of our weigh cells is the principle of Electromagnetic Force Restoration. The basis of principle is comparable to a simple beam balance. The weight is laid on one side of the beam. The result is that the coil attached to the other side of the beam tries to move out of the magnetic field of the magnet. The current is proportional to the force and is measured by a measurement resistor, transformed into a digital signal by an analogue -digital converter and further processed in a signal processing system. The object on the pan down with force equal to m* g, where 'm' is the mass of the object and 'g' is the acceleration due to gravity. The electronic balance uses the electromagnetic force to return the pan to original position. The electronic current that is required to generate the force is proportional to the mass that is displayed on the digital read out. Electronic analytical scales measure the force needed to counter the mass being measured rather than using actual masses. As such they must have calibration adjustments made to compensate for gravitational differences. They use an electromagnet to generate a force to counter the sample being measured and outputs the result by measuring the force needed to achieve balance. Such measurement device is called electromagnetic force restoration sensor.

Weighing Balance

Autoclave

Autoclave-

Boiling point of water is directly proportional to the pressure when the volume is constant. When the pressure is increased in a close vessel the temperature increases proportionally for example - at 15 lbs/sq.inch, temperature inside the autoclave is 121.6' C. This pressure & temperature is kept constant for 20 mins during autoclaving. It is sufficient to kill all the vegetative and spores of the organisms.

Hot Air Oven-

Hot air oven is mainly used for the following purposes:

1. Dry sterilization of syringes and needles (Between 140°C-160°C)

2. Preparation of anticoagulated bulbs.

3. Drying of glassware.

4. Heating of chemical used for the preparation of primary standards.

Hot Air Oven

When electricity is passed though the heating coils, electrical energy is converted to heat energy. The temperature is controlled by thermostat.

Incubator-

Double walled cabinet of mild steel, logged with insulation material

The inside door is made of glass panel set in metal frame and fitted with gasket.

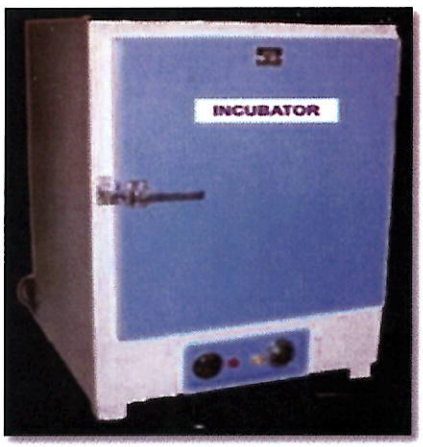
The out door is double walled and made up of mild steel.

Adjustable shelves are of crimped wire mesh.

The heating elements are fitted at the base of the incubator arranged with the efficient thermostat. The ventilation arrangement to allow passage of expanded inner air.

The temperature can be controlled by using a control knob, and it is recorded by a thermometer [temperature rang 30 °C-80 °C).

Incubator



Microscope-

A microscope is an instrument used to see objects that are too small to see naked eye. Microscopy is the science of investigating small objects and structure using such instrument. The microscope is an instrument which is absolutely essential for microbiologists. It is a combination of senses so adjusted that minute objects invisible to the naked eye are magnified and made visible. The term microscope is form from Greek word 'micro' small and 'scope' to view.

Microscope

Colorimeter-

Colorimetric analysis is a method of determining the concentration of a chemical element or chemical compound in a solution with the aid of colour reagent.

Principle of Colorimeter:

The working of colorimeters is mainly based on the Beer-Lambert's Law. This law states that the light absorption when passes through a medium are directly proportional to the concentration of the medium. When a colorimeter is used, there is a ray of light with a the working of colorimeters is mainly based on the Beer-Lambert's Law. This law states that the light absorption when passes through a medium are directly proportional to the concentration of the medium. When a colorimeter is used, there is a ray of light with a certain wavelength is directed towards a solution. Before reaching the solution the ray of light passes through a series of different lenses. These lenses are used for navigation of the colored light in the colorimeter. The colorimeter analyzes the reflected light and compares with a predetermined standard. Then a microprocessor installed in the device is used for calculation of the absorbance of the light by the solution. If the absorption of the solution is higher than there will be more light absorbed by the solution and if the concentration of the solution is low then lights will be transmitted through the solution.

Generally Beer's law is written as:

$$A \alpha c.l$$

where A-amount of light absorbed by the solution
c-concentration of solution
1-length of light path

Beer Lambert's Law:

Beer's Law

According to Beer's law when monochromatic light passes through the colored solution, the amount of light transmitted decreases exponentially with increase in concentration of the colored substance.

$$A \alpha c$$

where A-amount of light absorbed by the solution
c-concentration of solution

Lambert's Law
According to Lambert's law the amount of light transmitted decreases exponentially with increase in thickness of the colored solution.

$$A \alpha l$$

where A-amount of light absorbed by the solution
l-length of light path

Therefore, together Beer-Lambert's law is:

$$A \alpha c.l$$

where A amount of light absorbed by the solution
c-concentration of solution
l-length of light path

Working of colorimeter:
1]Source: Material that will go in excited state after giving high voltage or electric supply is used in colorimeter.
To generate visible light tungsten lamp is used which gives wavelength of 350-2500mm
To generate UV light H2/deuterium lamp is used.

2] Wavelength selector: It will produce monochromatic light from polychromatic light coming from source. There are two wavelength selectors:

i)Filter
Principle: filter will absorb all wavelength of light, except one which has to fall onto the sample. E.g.- Gelatin filter, Tinted glass filter.

ii)Monochromator:
Principle: dispersion of light into its component wavelength selection of single wavelength that has to fall on to the sample Maintenance of energy at detector level, when there is no sample in the sample holder
E.g. Prism monochromator, Grating monochromator.

3]Sample holder: Sample under study is kept in a glass tube, which is called as Cuvette.
Cuvette should satisfy certain criteria, which are as follows;
1)It should be phototransparant.
2)It should be even in diameter.
3)It should perfectly fit inside sample holder.

4]Detector:
Principle: Monochromatic light coming from wavelength selector will fall on to the sample, where the light may get reflected, absorbed by the sample or transmitted through the sample.
E.g. of detector:
1)photocell or photovoltaic cell
2)phototube or photo emissive tube

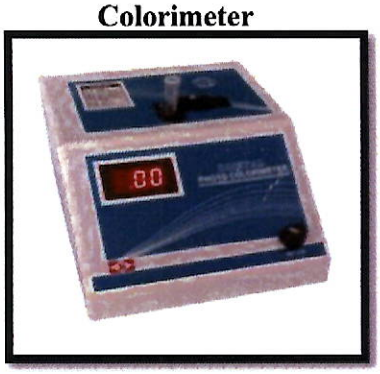
5]Amplifier:
Detector will develop current that will amplify by the amplifier. Amplifiers that are used include potentiometer and potentiometric recorder.

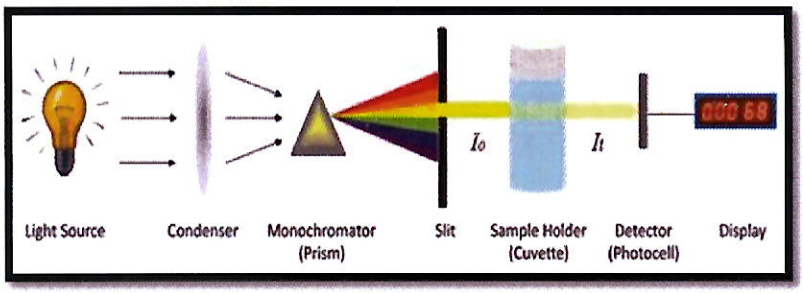
6] Readout signal:
A device which will indicate numerical value for the light absorbed by the specimen.

Applications of colorimeter:

1]Qualitative analysis
2]Quantitative analysis.
3]Molecular weight.
4]Control of purification or to check purity of sample.
5]To check cis and trans isomers.
6] For physiological studies also colorimeter is used.

Colorimeter





McFarland Standard-

McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing. An example of such testing is antibiotic susceptibility testing by measurement of minimum inhibitory concentration which is routinely used in medical microbiology and research. If a suspension used is too heavy or too dilute, an erroneous result (either falsely resistant or falsely susceptible) for any given antimicrobial agent could occur.

Original McFarland standards were made by mixing specified amounts of barium chloride and sulfuric acid together. Mixing the two compounds forms a barium sulphate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4).

Now there are McFarland standards prepared from suspensions of latex particles, which lengthens the shelf life and stability of the suspensions. The standard can be compared visually to a suspension of bacteria in sterile saline or nutrient broth. If the bacterial suspension is too turbid, it can be diluted with more diluent. If the suspension is not turbid enough, more bacteria can be added.

McFarland Standard No.	0.5	1	2	3	4
1.0% barium chloride (ml)	0.05	0.1	0.2	0.3	0.4
1.0% sulfuric acid (ml)	9.95	9.9	9.8	9.7	9.6
Approx. cell density (1X10^8 CFU/mL)	1.5	3.0	6.0	9.0	12.0

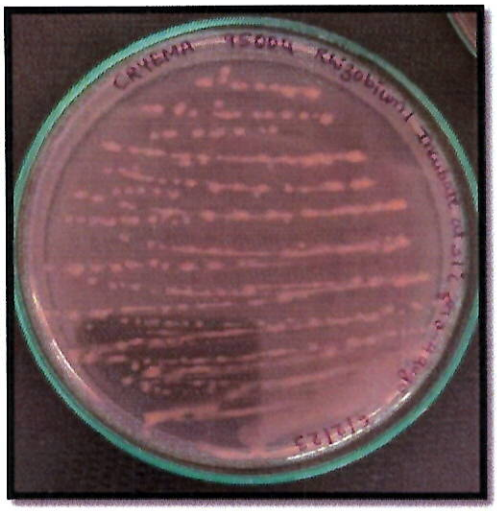
Reference:

Cockerill, Franklin R.; et al. (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. CLSI. p. 12. ISBN 978-1-56238-784-6.

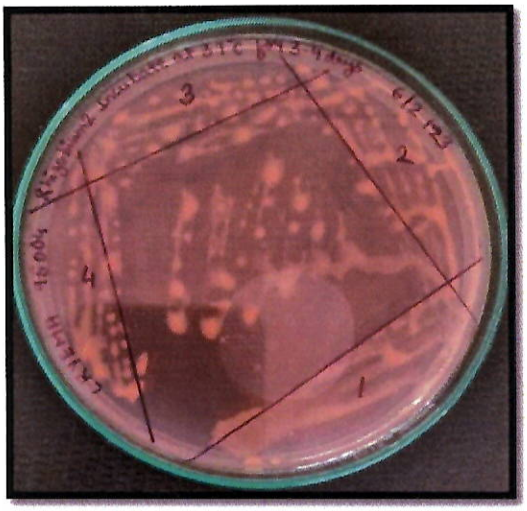
- **THE NEPHELOMETER: AN INSTRUMENT FOR ESTIMATING THE NUMBER OF BACTERIA IN SUSPENSIONS USED FOR CALCULATING THE OPSONIC INDEX AND FOR VACCINES. JOSEPH McFARLAND.**

OBSERVATION

1] Colonies observed in Congo Red Yeast Extract Mannitol Agar Plate [CRYEMA] :-

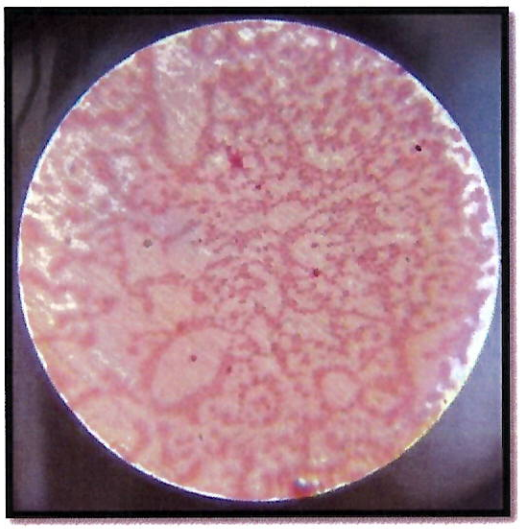


For Sample 1

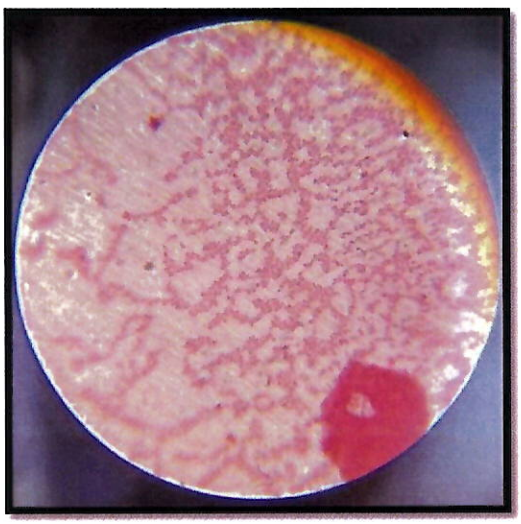


For Sample 2

2] Gram Staining of Colony picked under Microscope (100× lens) Oil Immersion:-



Gram Negative Rods [Sample 1]



Gram Negative Rods [Sample 2]

Colony Characteristics:-

Observation	Sample 1	Sample 2
Size	2-3 mm	2 mm
Shape	Circular	Circular
Colour	White	White
Elevation	Convex	Convex
Consistency	Smooth	Smooth
Opacity	Translucent	Translucent
Margin	Entire	Entire
Gram's Nature	Gram Negative Rods	Gram Negative Rods

Biochemical Tests:-

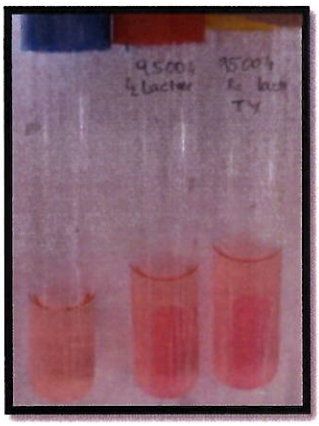
Observations	Sample 1	Sample 2
Sugar Tests		
1% Glucose	+ve	+ve
1% Maltose	+ve	+ve
1% Mannitol	+ve	+ve
1% Lactose	+ve	+ve
IMVIC Tests		
Indole Test	+ve	-ve
Methyl Red Test	+ve	+ve
Vogus Proskauer Test	+ve	+ve
Citrate Test	+ve	+ve
Triple Sugar Ion Slant	+ve	+ve
Urease Test	+ve	+ve
Catalase Test	+ve	+ve
Starch Hydrolysis Test	+ve	+ve
Glucose Peptone Agar Plate	-ve	-ve

Sugar Test:-

1% Glucose

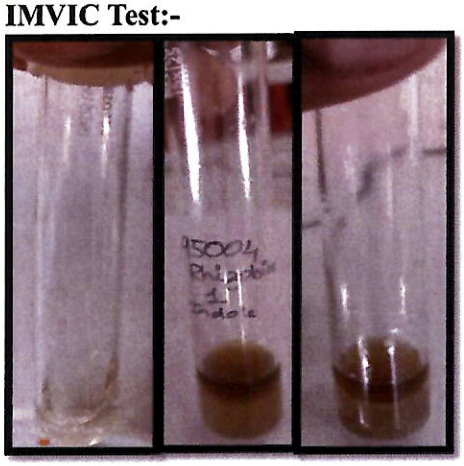
1% Maltose

1% Mannitol

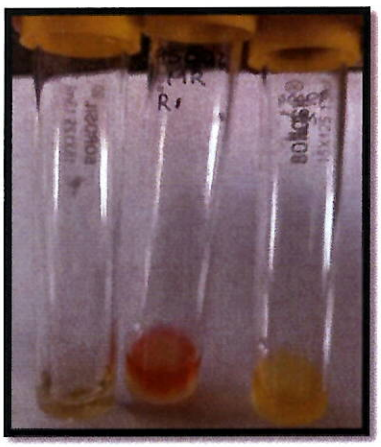


1% Lactose

IMVIC Test:-



Indole Test



Methyl Red Test

Vogus Proskauer Test

Citrate Test

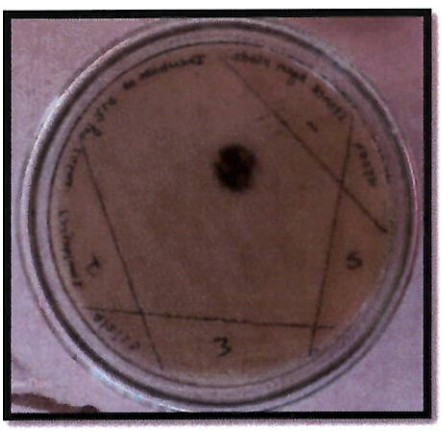
Other Tests:-

Triple Sugar Ion Test

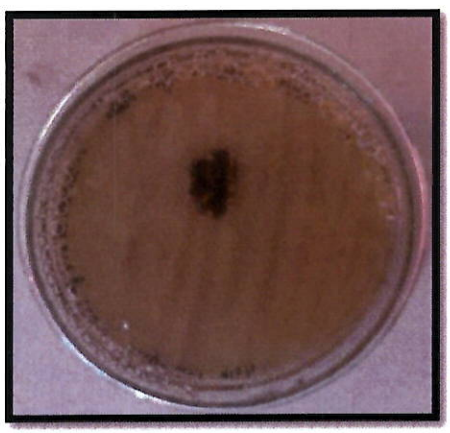
Urease Test

Catalase Test

Starch Agar Plate:- Positive results after adding Lugol's Iodine reagent



Sample 1

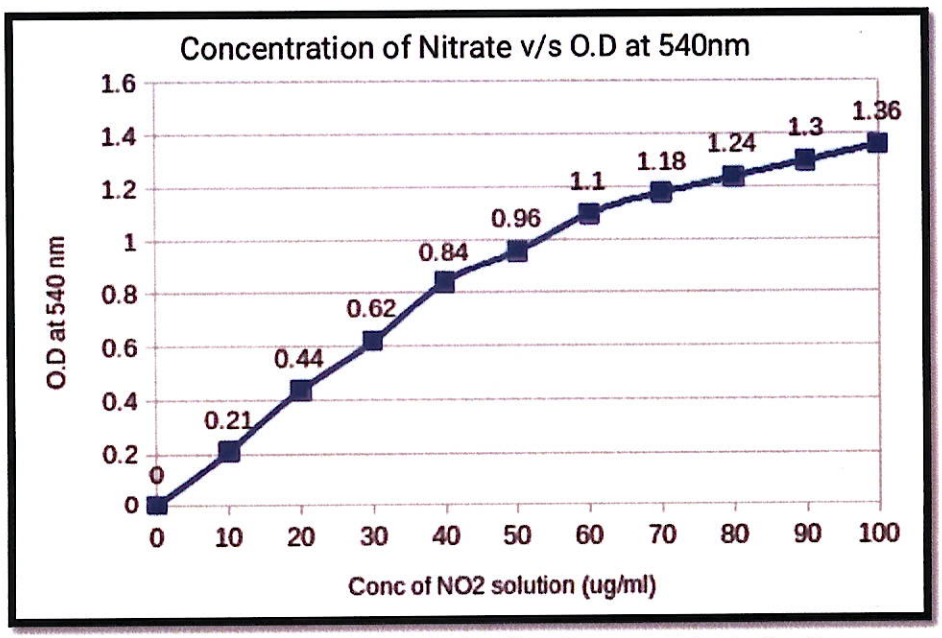


Sample 2

The Standard Concentration Table:-

Dilution Scheme:- Stock:- Nitrate solution (100µg/ml)
Diluent:- Distilled water
Reagents:- Sulphanilamide solution
NEDD solution
OD:- 540nm

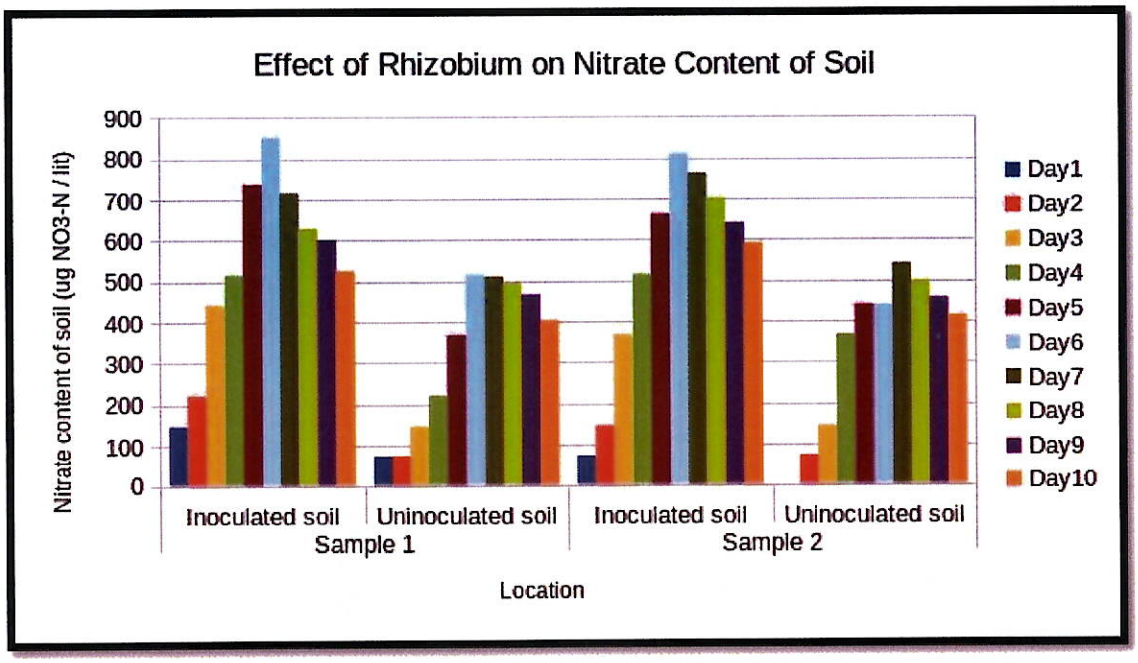
Sr. No.	Concentration of Standard (µg/ml)	Amount of Standard(ml)	Amount of Diluent(ml)	Total volume (ml)	Sulphanilamide Solution(ml)	NEDD (ml)	OD at 540nm
1	Blank	0	10	10	0.5	0.5	0.0
2	10	1	9	10	0.5	0.5	0.21
3	20	2	8	10	0.5	0.5	0.44
4	30	3	7	10	0.5	0.5	0.62
5	40	4	6	10	0.5	0.5	0.84
6	50	5	5	10	0.5	0.5	0.96
7	60	6	4	10	0.5	0.5	1.10
8	70	7	3	10	0.5	0.5	1.18
9	80	8	2	10	0.5	0.5	1.24
10	90	9	1	10	0.5	0.5	1.30
11	100	10	0	10	0.5	0.5	1.36



Graph1:- Standard line graph for the nitrate content of soil

Table representing the Nitrate content of soil after calculating the reading with formula which is as follows:-

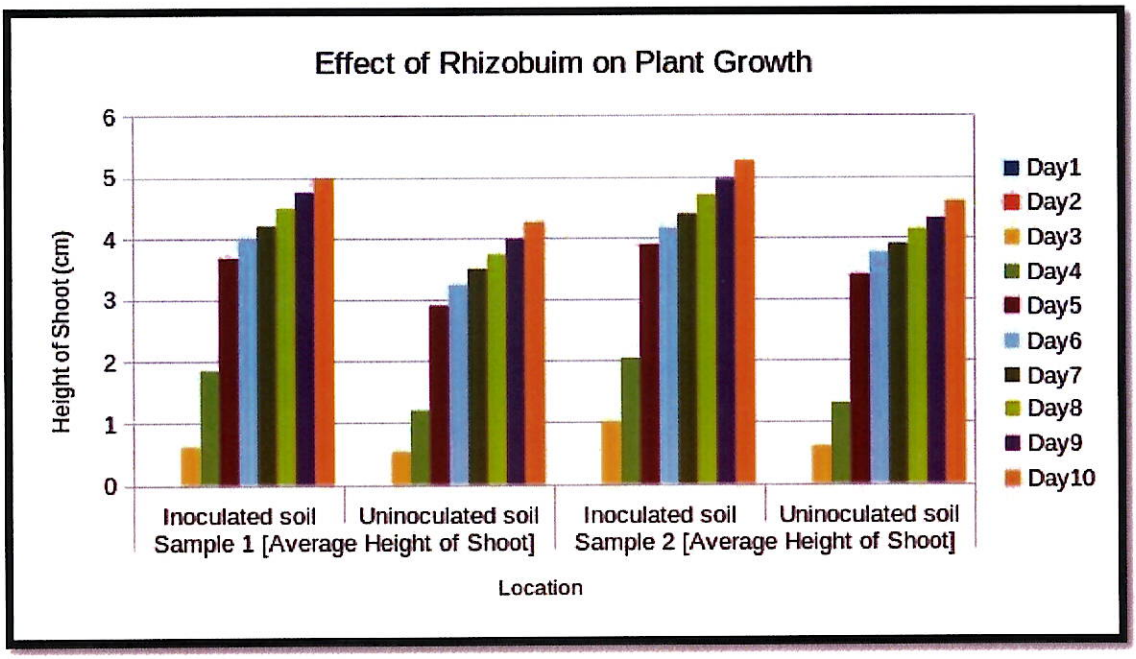
Days	Kosbad soil Sample 1		Kosbad soil Sample 2	
	Inoculated	Uninoculated	Inoculated	Uninoculated
1	147.05	73.52	73.52	0.0
2	220.58	73.52	147.05	73.52
3	441.17	147.05	367.64	147.05
4	514.70	220.58	514.70	367.64
5	735.29	367.64	661.76	441.17
6	850.79	514.70	808.82	441.17
7	715.39	509.77	760.66	540.78
8	628.96	493.64	701.23	497.59
9	601.25	465.33	640.79	456.91
10	523.76	402.37	590.86	413.67



Graph 2:- Rhizobium effect on soil nitrate content

Plant Growth due to effect of Rhizobium:-

Day	Kosbad Sample 1 [Average Height of shoot]		Kosbad Sample 2 [Average Height of shoot]	
	Inoculated	Uninoculated	Inoculated	Uninoculated
1	-	-	-	-
2	-	-	-	-
3	0.6	0.52	1.00	0.60
4	1.85	1.19	2.04	1.3
5	3.68	2.89	3.89	3.40
6	4.00	3.24	4.16	3.76
7	4.20	3.50	4.38	3.90
8	4.48	3.73	4.69	4.14
9	4.74	3.98	4.95	4.31
10	4.97	4.25	5.25	4.58



Graph 3:-Effect of Rhizobium on plant growth

Sample 1

Sample 2

RESULT AND DISCUSSION

Isolation of bacteria from Root Nodules:-

The root nodules of Fenugreek plant was isolated and identified to be Gram Negative Rod for both the samples collected from the region.

The colony characteristics of the isolates showed white, circular, translucent, convex colonies on the CRYEMA plate showing presence of Rhizobium bacteria.

Biochemical test and other tests:-

All the fermentable sugars used for the sugar test showed positive results and indicates that the bacteria utilize the sugar as a source of energy.

IMVIC test performed for both the samples showed positive results.

Other test includes the Catalase test which showed effervescence after reacting with hydrogen peroxide and showed positive results, Urease test performed showed positive results, Starch hydrolysis test showed positive results after addition of lugol's iodine and Glucose peptone agar test showed no growth of isolates.

Effect of Nitrate content of soil:-

The nitrate content in soil showed gradual increase as the rhizobium sample was inoculated in the soil.

Both the samples which were inoculated showed high nitrate content comparative to the soil with no rhizobium culture.

The nitrate content in the soil showed decrease as the plant started to utilize it for its growth

Resulting that the Rhizobium culture inoculated shows effect on the nitrate content and helps the growth of plant.

Plant growth due to effect of Rhizobium:-

The Fenugreek seeds sown for both samples as inoculated and uninoculated, where the growth of the Fenugreek plant showed growth after the 4^{th} day for both the samples in inoculated the growth was more than inoculated sample.

CONCLUSION

The isolation of Rhizobium bacteria was been done by using CRYEMA plates for both samples were observed after the incubation the white, circular, translucent colonies were been observed further the Grams staining showed the Gram negative rods and the isolates of the both samples used in test by using Sterile Glucose Peptone Agar plates there were no colony growth observed indicating that the root nodule samples of Fenugreek plants showed the presence of Rhizobium bacteria in it which was been collected from areas of Kosbad village near Dahanu takula, Palghar District.

The both samples brought from nearby regions of Kosbad were been examined for biochemical test were both the sample were inoculated for sugar test which were able to use the sugars that is 1% Glucose,1% Maltose, 1%Mannitol, 1% Lactose which is utilized as the source of energy. Further test performed namely IMVIC in which the Indole test showed that the isolate can synthesize the tryptophanase enzyme it is an acid and can also utilize citrate showing positive results. There were some other test performed were the isolates had the tendency to show acid production and effervesces in catalase test as a positive result , they also had synthesize amylase which is observed in starch hydrolysis test and the isolates also showed positive response for urease test.

To check the nitrate content of soil both the soil samples of inoculated was found to be higher than the uninoculated soil. After comparing the sample 1 and sample 2 the sample 1 showed higher nitrate content than the sample 2 here the nitrate content of soil was observed up to 10 days in which it showed rise up to 6 days then there was an decrease in the nitrate content level in soil because as the nitrate content was increasing the plant took nitrate for its growth there was an decrease in level of nitrate content. As the further being observed it was been was found that the plants growth for inoculated soil both the sample was higher than the uninoculated soil. As this plant growth seen higher in inoculated soil helps to conclude and gets in consideration that the Rhizobium is going to show positive effect of growth of plants.

FUTURE PROSPECTS

As the increasing and developing global market for biofertilizers there is an increase in organic farming. Further for checking other methods DNA sequencing of plants is done for changing the gene expression for future development to deal with different stressed conditions and diseases. Thus, such upgrowing methods in upcoming years will help for use of biofertilizers in every single farming which will be beneficial as per reduce in pollution and to get chemical free and naturally by organic matter based crops and which also be helpful in the better production of yields. Further even the antimicrobial activity of Rhizobium species strains can be studied against pathogenic organisms and fungus which are harmful for plants and also a comparative study between Rhizobium and Azotobacter to check the nitrogen fixing capacity from the same soil.

REFERENCE

1] Datta A, Singh R K and Kumar S. 2015.Isolation, characterization, and growth of Rhizobium strains under optimum conditions for effective biofertilizer production. International Journal of Pharmacology Science 32: 199-08.

2] Deshwal V and Chaubey A. 2014. Isolation and characterization of *Rhizobium Leguminosarum* from root nodule of *Pisum sativum* L. Journal of Academia and Industrial Research 2: 464–67.

3] Gault, R.R., Byrne, P.T. and Brockwell, J. (1973) Apparatus for surface sterilization of individual legume root nodules. Lab. Pract. 22, 292-294.

4] McFarland J (1907). Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc 14:1176-1178.

5] Pawar V A, Pawar P R, Bhosale A M and Chavan S V. 2014. Effect of Rhizobium on seed germination and growth of plants. Journal of Academia and Industrial Research 3:84-88.

6] Peoples M B, Herridge D F, Ladha J K. Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production. Plant Soil. 1995;174:3–28.

7] Singleton, P.W. and Tavares, J.W. (1986) Inoculation response of legumes in relation to the number and effectiveness of indigenous rhizobium populations. Appl. Environ. Microbiol. 51, 1013-1018.

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https://www.researchgate.net/publication/318642628_Isolation_and_Characterization_of_Rhizobia_from_Rhizospher_and_Root_Nodule_of_Cowpea_Elephant_and_Lab_Lab_Plants

https://www.researchgate.net/publication/350102587_Growth_and_yield_of_fenugreek_Trigonella_foenum_graecum_L_in_response_to_different_levels_of_phosphorus_and_biofertilizer_Rhizobium_and_PSB_under_Kymore_Plateau_and_Satpura_hill_agro-climatic_zone_of_

https://labmonk.com/isolation-of-rhizobium-from-root-nodule